THE SOLUBILITY OF CALCIUM SALTS

STANFORD UNIVERSITY LIBRARIES

BY

JULIUS SENDROY, JR.

L.C.

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE, COLUMBIA UNIVERSITY

STANFORD UNIVERSITY LIBRARIES
WITHDRAWN

NEW YORK
1927

3781
C72S
THESIS
Dissertation

THE SOLUBILITY OF CALCIUM SALTS

BY

JULIUS SENDROY, JR.

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE FACULTY OF PURE SCIENCE, COLUMBIA UNIVERSITY

NEW YORK
1927

Solubility of calcium carbonate and tertiary calcium phosphate in serum.
Introduction.
Experimental.
Horse serum shaken with $CaCO_3$ and $Ca_3(PO_4)_2$ for varying lengths of time.
Horse blood shaken with $CaCO_3$ and $Ca_3(PO_4)_2$ for varying lengths of time.
Effect of shaking untreated horse serum at varying $p\alpha_{H^+}$.
Horse serum shaken with $CaCO_3$ and $Ca_3(PO_4)_2$ at varying $p\alpha_{H^+}$.
Horse serum with added $Ca(HCO_3)_2$ shaken with $CaCO_3$ and $Ca_3(PO_4)_2$ at varying $p\alpha_{H^+}$.
Horse serum shaken with $CaCO_3$ and $Ca_3(PO_4)_2$ after the addition of excess phosphate.
Horse serum rotated at 38° after addition of excess phosphate.
Variation of calcium concentration with dilution of serum after shaking with $CaCO_3$ and $Ca_3(PO_4)_2$.
Variation *in vitro* of $pCaCO_3$ and $pCa_3(PO_4)_2$ with $p\alpha_{H^+}$ in serum of normal and pathological humans.
Solubility of calcium salts *in vivo* in serum of pathological humans.
Discussion.
Bibliography.
Vita.
Publications.

THE SOLUBILITY OF CALCIUM SALTS.

INTRODUCTION.

The biological importance of the element calcium has been recognized since the earliest times, but it has only been comparatively recently, through the stimulus afforded by the growing realization of the close association between some pathological conditions and disturbances of the calcium metabolism of the body, that the element calcium has claimed so much attention and study in biological fields. Being the only cation, except for small amounts of magnesium, capable of forming difficultly soluble salts with the common anions present, namely carbonate and phosphate, it carries unaided the burden of furnishing the organism with its framework of ossified tissue, the skeleton. However, its function and purpose do not cease here. Its effect on nerve and muscle permeability and irritability alone would warrant most important consideration of the nature of its metabolism.

It has almost always been found that irregularities in the calcium metabolism of the body have been associated with some variation from the normal in the physiology of the system. The tendency has been to regard the pathological condition as the *result* of the calcium variation. However, it is sometimes most difficult to establish the causal relationship between the two, and the attempt to relate pathology and calcium always as effect and cause respectively, has, no doubt, in some cases been the obstacle to further explanation. It may be that some pathological conditions are *not* the result, but the *cause* of calcium metabolism disturbances.

Being such an important element, the behavior of calcium in the distributing medium, the blood stream, would naturally be the subject of much interest and study. It is well known that the level of calcium in the blood is one of the most constant of any of the substances in the entire animal organism. Hence, one would expect that any departure from the normal level would be accompanied by some definite variation from normal

conditions, in some portion of, or in the function of, some organ or organs, of the body. Evidently the blood calcium level is regulated by some biophysical or biochemical mechanism, to remain as nearly as possible at a concentration which is the optimum for the organism under all conditions. However, it was not until Rona and Takahashi (1911) calculated that the amount of calcium in blood was far in excess of that which might be held in solution by simple salt solutions of the same carbonate and phosphate content at the same hydrogen ion concentration, that it was brought to the attention of the biological world that the regulation and the regulator of the calcium level in blood were of paramount importance as another example of the biological adaptation of the blood stream to fit the requirements of the body. It must be pointed out here, that while a study of the blood calcium, or more conveniently of the calcium of serum, which holds most of the blood calcium, is a most important index of calcium metabolism in the body, a complete explanation of the physiology involved in the regulation and distribution of that element can never be obtained without experimentation with the actual intact organism. In other words, a study *in vitro* of the chemical, physical, and thermodynamic properties of serum, with respect to its calcium content, cannot possibly furnish a complete analogy to the behavior or properties of this element in the organism. When removed from the body, it may be expected that some of the factors contributing to its regulation and particular behavior may be lacking.

On the other hand, realizing this limitation, one may study *in vitro* the nature of the substances that may be involved, and so obtain some idea as to what extent one may explain the behavior or properties of serum calcium on the basis of the behavior of that element or radical under conditions, and in systems, which are well known. It was with this idea, and not with that of furnishing a complete explanation for the behavior of calcium in serum of the body, that the present work was undertaken. As will appear later, comparative studies of calcium in salt solutions and in biological fluids, under certain conditions, furnish us only with a suggestion as to what may be taking place, in a similar way, in the body. However, some phenomena previously unknown, or incapable of explanation, have been revealed.

Concerning calcium metabolism as a whole, we must bear in mind the physiological picture. The organism has a large reserve

of calcium in the bones as calcium carbonate and calcium tertiary phosphate. The circulating system contains a constant concentration of calcium and is the intermediary between the calcium of the bones and tissues and the calcium in the alimentary canal taken in the food. There is very little, if any, absorption of the digestive products from the stomach, most of it taking place through the intestinal wall. However, it is known that excretion may also take place through the gut, so that all other conditions being the same, if the level of serum calcium is maintained, almost as much calcium should be excreted in the feces and urine under *normal conditions*, as is ingested in the food. Metabolic studies have shown that regardless of what proportion of the food calcium over and above the normal requirement may actually be absorbed, the calcium balance remains almost constant in the adult organism under normal conditions, the rate of further storage of this element being very slow.

In time of stress or demand for calcium, however, as in pregnancy, lactation, growth, healing of fractures, etc., the calcium balance must become positive by an excess of intake over output; or else, as in starvation or with a deficient food diet (Sherman, 1921) the calcium reserve of the body, mostly that of the bones, will suffer a loss of calcium. The point is, that under most of these conditions the calcium in serum remains at an almost uniformly constant level, being a little higher during the period of growth and development. The serum being practically unchanged with respect to its other constituents, the serum calcium level of the normal adult will be maintained, no matter what the absorption, excretion, or withdrawal from the bones and tissues may be. Such constancy in a system which would be expected to be changed in one direction or the other by, and in response to, external influences, is a most certain indication of a physicochemical equilibrium. The question suggests itself: Is there some specific organ or set of organs regulating this serum calcium level, or controlling the equilibrium of absorption and excretion or precipitation and solution, or is the concentration maintained as a result of the chemical, physical, and thermodynamic properties of the serum itself?

Recent work has been stimulated by the interest in this and similar questions, the result being that evidence has been brought forward that the regulation of the *serum calcium level* may be influenced by both factors. The experiments reported in these

papers, in so far as they are studies of serum calcium behavior and properties, may be taken as an indication of the extent to which serum itself may be regarded as a factor in the calcium equilibrium.

Nature of the Calcium Compounds of Serum.—The literature on the subject is so voluminous, and so well known, that it is unnecessary to quote specific references or authorities to state that the problem of the behavior of serum calcium is centered about the question of the different forms in which the calcium may be present, and in the quantitative relationships that may exist between such forms. A quantitative investigation such as this, it was hoped, would throw light on the factors influencing the functional activity of calcium in serum and in the organism.

In considering the possible nature of the calcium compounds of blood serum, it may be advantageous to indulge in a cursory review of some substances known to be present in blood under normal conditions and the possibility of calcium being combined with any of these substances. This inquiry may be made from the standpoint of their chemical and physical properties and that of serum itself, and from a consideration of what does or does not happen to these substances and to calcium *in vitro* and *in vivo* under various conditions.

In such a system as blood, one might reasonably inquire whether calcium may be present in solution as the carbonate, bicarbonate, tertiary, secondary, and primary phosphate, chloride, sulfate, and as a compound of protein, some amino acids, fatty acids, lipoids such as lecithin, as the salt of hexosemonophosphoric acid, or as a compound of any other substance or substances that may be slightly dissociated, such as citric acid. Evidently, the total calcium that could be carried in solution in such a system would be governed by whether the bone salts of serum were in equilibrium with bone or not, by the solubility in serum of the calcium salts present at physiological pH, by the amount that could be present in solution in undissociated form, and by the amount that could be held by some means or other in physical suspension.

Of the inorganic salts of calcium in the organism, there are three difficultly soluble ones. Bicarbonate and phosphate salts exist in serum, while $CaCO_3$ and $Ca_3(PO_4)_2$ are found in the bones. The bone salts must be transported to their destination by the blood stream, which, if it does not reach the bone cells directly, can at least supply calcium through the medium of the tissue

fluids. Calcium carbonate, secondary phosphate $CaHPO_4$, and tertiary phosphate $Ca_3(PO_4)_2$, are the difficultly soluble salts of calcium in the body, the latter being by far the most insoluble.

Rickets has been shown to be associated with either low serum phosphate or low serum calcium. From this alone, one would gather that rickets is caused by the failure of calcium salts to deposit in osseous tissue, due to a lowered solubility product for $Ca_3(PO_4)_2$. This has been the idea advanced by Howland and Kramer (1922, 1923), and more recently by Holt (1925) in connection with his theory of supersaturation.

If we assume that the serum calcium exists only in the form of salts of the inorganic acids of serum, we are committed to one or more of three possible theories as to the means whereby so much calcium may exist in serum: (*a*) that the solubility of these inorganic salts is increased due to the presence in serum of a substance (or substances) which exerts marked specific ion effects, or changes the dielectric constant of the solute; (*b*) that infinitesimally small particles of these salts exist in solid colloidal form, either distributed throughout the serum, or adsorbed onto the proteins; (*c*) that the calcium salts are in the metastable equilibrium of supersaturation.

Taking up the question of the inorganic calcium, some of the evidence given in these papers and in the work of Holt, La Mer, and Chown (1925), would tend to substantiate the idea that serum has a specific ion effect on the solubility of calcium salts. Inasmuch as we have no direct method for measuring Ca^{++} ion activity, this idea can neither be wholly rejected nor accepted. The idea of specific ion effect should only be used when all other attempts at a possible explanation have failed. As shown later we can be fairly certain that some of the protein effect, at least, can be attributed to the base-binding power of this class of substances.

Concerning the idea that colloidal calcium may exist in solution and remain unprecipitated for days, we have the evidence of Watt (1923) whose work seems to have escaped notice and deserves more than passing mention. He performed extensive microscopic studies of the precipitation of calcium carbonate and phosphate under varying conditions and in different media. The phosphate always precipitated in fine amorphous granules, which gathered in clumps and masses and fine films. Confirming the work of others, he reports that the presence of colloids seemed to have no influence on the precipitation of phosphate, which

always came down in fine amorphous granules and gathered in clumps and masses. He did not determine whether secondary or tertiary phosphate was obtained. Calcium carbonate precipitated in water and salt solutions to yield, as the final stage, a mass composed only of crystals. In colloidal substances, however, there was a marked tendency for the precipitate to persist in the form of spherules, or flattened discs, called calcospherites. Brownian movement in some cases persisted for even a year, due to the colloidal material retaining some of the precipitate in its original extremely fine granular form, thus preventing coalescence and formation of larger particles. When spherules did collide, they united to form crystals of glassy hardness. In areas where precipitation was denser and more rapid, larger spherules were formed.

In salt solutions, precipitation was much more rapid, and all forms were crystalline. Urea aided the formation of spherules, even in aqueous solution, as did lecithin, which exercised a typical colloidal action on the precipitate, favoring the large spherical forms of deposit. Watt's opinion is that these substances may be involved in calcareous incrustation of sclerotic arteries and in the development of other pathological calcifications.

It may be significant that he noticed that ammonium carbonate caused great clumping and fusion of calcium carbonate. It has been suggested that bone formation is a result of precipitation in osseous tissue due to ammonia formation.

Hyaline cartilage extract was found to retain excessive amounts of calcium without precipitation. The cartilage itself was unfavorable to the deposition of calcium salts. In fresh bone of various animals, examination revealed that the matrix around the bone cells was homogeneous, transparent, almost glassy in appearance, and gave no indication of being formed of separate particles. If originally so, there had been complete fusion and no evidence of precipitation remained. Hence, this work and that of others makes it seem that calcium is never deposited directly in the cartilaginous matrix, but that the cartilage preceding the bone is destroyed and replaced by the organic bony matrix, which is of a fibrous character, and that this has the calcium salts deposited in it. Watt believes in Barillé's (1910) specific calcium carbonophosphate compound in blood, which precipitates the salts always in the same proportion in bone. That this is not a necessary hypothesis for constancy of bone composition,

must be admitted when we remember the particular constancy of calcium, and the general constancy of phosphate and CO_2 in the blood stream, at the same given points. Furthermore, the idea of bone formation being a precipitation caused by change of reaction due to ammonia formation or loss of CO_2, would cause the necessity of the corresponding idea that an acidosis is necessary in order to be able to draw upon the bone reserve. True, there is an acidosis in starvation, and Goto (1918) found a depletion of calcium carbonate in bones of rabbits in experimental acidosis, but these may be considered as incidental phenomena, not necessarily related to the *need* for calcium. The acidosis of pregnancy would seem to be a way of reversing bone formation due to a *need* which is not met through absorption. Such an acidosis may not necessarily be for the purpose of dissolving calcium salts directly, but may be the favorable reaction which influences the bone cells in yielding calcium to the blood stream.

Recently, Holt, La Mer, and Chown have advanced the theory that serum is supersaturated with tertiary calcium phosphate, and that the maintenance of a definite level of supersaturation is necessary to avoid pathological conditions such as tetany and rickets, associated with an insufficiency of calcium in the blood. They saturated serum with solid $Ca_3(PO_4)_2$ and obtained a considerable decrease in total calcium content, though not to serum salt level, however, and ascribed their results to supersaturation. As will be shown later, examination of the phosphate figures of their own data, also of Holt's (1925) experiments on cerebrospinal fluid, from which he draws the same conclusions, reveals that the amount of calcium precipitated was far in excess of that required to precipitate $Ca_3(PO_4)_2$ or $CaHPO_4$. Furthermore, the difference in level of the stoichiometric solubility products of $Ca_3(PO_4)_2$ of serum and cerebrospinal fluid can only mean that a corresponding amount of extra calcium is accounted for by either the specific ion effect of the proteins on solubility, or by a union of calcium with them. Since cerebrospinal fluid contains no appreciable amount of protein, the apparent explanation is that a diffusible, inactive calcium compound is present.

It has been shown by Watt and others that adjacent tissues, apparently similar, may exist together, and one be calcified and the other not. Evidently the salt precipitation or deposition in tissue is dependent on *some specific property* of the tissue or of some substance in it, and not solely on the concentration of the

mineral elements *in the serum* of the blood reaching the bone. Further evidence in favor of this idea has recently been furnished by Bosányi (1925), who has been able to cure rickets by feeding hemoglobin and injecting extracts of bone marrow. He ascribes the activity of these substances to their histone-like character, and suggests that arginine may be the factor in bone marrow, the deficiency of which causes poor calcification. According to him, recalcification of rachitic bone is a primary condition which takes place as a consequence of a local change within the tissue itself. The augmentation of the inorganic phosphorus of the serum would seem to be a secondary sequella of the local calcification. The latter statement would seem to be substantiated by one of his cases, in which an advanced degree of healing took place *in vivo* in rachitic bone at a time when the patient's blood serum showed a low phosphorus of 2.0 mg. per cent, with calcium 9.6 mg. per cent.

Bearing on the same question are the results of Shipley, Kramer, and Howland (1925), who performed *in vitro* calcification experiments with rachitic bones. They found that such bones did not calcify in rachitic serum, but did in normal serum. Calcification was also obtained when the initial concentration of calcium and phosphates in salt solutions approximated that of serum, but was not lower. Egg albumin prevented calcification, heat and protoplasmic poisons inhibited it. Evidently, here also, we have an example of the fact that calcification is not a simple process of precipitation, but depends not only on the calcium and phosphate supply, but also on the integrity of the bone tissue and of the blood serum.

If we wish to consider the possibility of the apparent excess of calcium in serum being held therein in combination with some substance other than inorganic, we must examine the list of organic serum constituents to rule out if possible, those that are known to be incapable of binding calcium in serum. It will help, perhaps, to lay stress on such substances as may be involved, by synthesis or destruction, in calcification processes.

At first glance, the idea of calcium soaps may seem attractive, but it has been shown by Wells (1906) that such soaps are not a factor in calcification. Furthermore, the dissociation constants of these fatty acids preclude the possibility that calcium can exist in this form in serum.

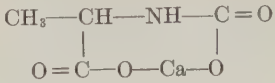
Concerning the possibility of calcium being bound to lecithin,

we know nothing. If such a union were possible, we would have a substance containing phosphorus and calcium in the same molecule. However, it would seem that if lecithin were a step in the process of calcification, its presence would have been detected in the work on calcification done by Wells and others, who looked for it, but obtained negative results.

It has been quite definitely shown that proteins, particularly albumin, have an affinity for calcium. Calcium carbonate is more soluble in a protein solution than in an aqueous solution containing the same amount of salt. Greenberg and Schmidt (1926), studying the transport numbers of alkali and alkali-earth casein compounds, have shown that part of the calcium of such protein solutions is bound by casein in non-ionic form, producing a complex which carries a certain amount of the element in a direction opposite to that of the cation. Other evidence seems very favorable for the existence of calcium protein compounds. Salvesen and Linder (1923) have demonstrated a direct relationship between protein and calcium in the serum of nephritic patients. Furthermore, as has been shown by unpublished work of Murray, Hastings, and Sendroy, one may calculate the amount of calcium bound per gram of protein in serum at different pH's. The equation

$$CaP = 0.01\,P(p\alpha_{H^+} - 6.0)$$

represents the quantitative relationship between calcium and total protein in serum. However, the total amount of calcium as salt and as protein compound cannot account for all of the calcium found in normal serum. Hence, we must look for some other substance capable of binding and holding calcium in solution. In this connection, it may be interesting to note that alanine may unite with CO_2 in the presence of a lime salt, to form the compound



by the carbamino reaction (Mathews, 1920). Carbamates are believed to exist in the body during the formation of urea from ammonia.

Robison and his collaborators (1922, 1923, 1924) have demonstrated the presence in blood of one or more hexosephosphoric esters which, interacting with an enzyme found in various tissues,

even in the bones of rachitic animals, can induce precipitation of calcium by hydrolysis of the ester. Such a substance can retain calcium in solution until the enzyme acts upon it and hydrolyzes it. Moreover, the optimum pH for this reaction is about that of blood. Lecithin, which may be considered a derivative of glycerophosphoric ester, is not hydrolyzed by the esterase. Robison's opinion is that after hydrolysis, calcification is just a matter of exceeding the solubility product of $Ca_3(PO_4)_2$.

Recently, Collip (1925) isolating the active principle of the parathyroid gland, has secured a substance, which upon injection, causes among other results an enormous increase in the serum calcium of both normal and parathyroidectomized dogs. Upon cessation of parathormone treatment, the calcium sinks back to its original level. At first it was thought that this substance acted by causing an increased absorption of calcium through the intestine, but Greenwald and Gross (1925) have shown that such is not the case. Furthermore, they believe that the active constituent of parathyroid extract causes a tendency to withdraw calcium from the bones. In this withdrawal, the parathyroid hormone is the important substance, either alone or as an accessory to some other substance, which can, by dissolving calcium from the bones, retain comparatively large amounts of calcium in solution. Tetany is to be considered as caused by the disturbance in the equilibrium between the inorganic calcium and the organic calcium, controlled by the parathyroid hormone.

Cameron and Moorehouse (1925), studying the calcium in serum and cerebrospinal fluid of parathyroidectomized dogs, have also come to the conclusion that tetany results because of the disturbance in equilibrium between inorganic and organic calcium. According to them, tetany is only indirectly caused by parathyroidectomy, being due to the decrease in inorganic calcium when some of it tends to go to the organic form to restore the normal equilibrium. There is a lowered inorganic threshold, and the decreased calcium ion concentration results in increased nerve and muscle irritability. The specific organic calcium compound of plasma is supposed to be less dissociable than the corresponding compound of serum into which it is transformed by some process of clotting. Their interpretation would seem to have one consequence, that clotting is not only an irreversible process with respect to fibrin, but also with respect to some specific organic compound of calcium.

Theoretical Considerations.

Much work has been done in recent years to show that the problem of the functional activity of calcium is really concerned with the various forms in which calcium may exist, and the relationships of the different forms involved. Apparently, calcification does not bear a simple relationship to the calcium of the blood. In other cases of calcium disturbance, the rise or fall of total calcium in serum is but a very poor index of what may really be happening. With the acquisition of new knowledge concerning the physiological behavior of calcium in the organism, it has become more and more apparent that we are concerned with several different forms of calcium, which may or may not be in equilibrium with each other, or exert influence upon each other.

The conception that the biological importance of calcium in cellular activity may be attributed to ionic calcium, is of fairly recent origin. This appreciation of the possible importance of the calcium ion in physiological systems has been the natural result of the development of the theory of solutions from theoretical considerations concerning the influence of the electronic environment upon the properties of ions in general. In recent years, various methods have been employed in an effort to accomplish a difficult task, the measurement of calcium ion concentrations in liquid systems in general, and in serum and biological fluids in particular. Many have attempted to obtain these values by the analysis of ultrafiltrates, some by electrometric measurements with amalgam electrodes (Neuhausen and Marshall, 1922), some by colorimetric methods, and others by calculation from experiments with difficultly soluble salts (Rona and Takahashi, 1911), (Kugelmass and Shohl, 1923-24), (Holt and collaborators, 1925). The latter method is the one which, in connection with the theoretical considerations outlined in the following, has been adopted for these studies.

Within the last few years a conception of ionic behavior has entered biological investigations, which relates the environment about the ions to the activity of the ions. Since this conception seems to be fruitful in the interpretation of studies involving electrolyte behavior generally, a brief review of the more important features will be given.

The failure of strong electrolytes to conform to the behavior predicted by the mass law, led G. N. Lewis in 1907 to propose a new thermodynamic concept called the *activity*, related to *fugacity* as concentration is to pressure; namely,

$$\text{fugacity} = RT \text{ activity}.$$

The fugacity may be regarded as the vapor pressure of a solute corrected for all deviations from the gas law, and hence is an accurate measure of the tendency of a substance to escape from its surroundings. In a similar way, by defining the activity of a substance as the ratio between the fugacity f of a substance in some given state, and its fugacity f° in some other chosen standard state, we have a quantity which is an accurate measure of the escaping tendency of a substance expressed in terms of concentration units which have been corrected for all deviations from the gas laws. By using these new units corrected for deviations of the substance in the gaseous state, from the behavior of an ideal gas, one may apply them to any system,

and yet obtain relationships which conform to those derived on the supposition that the gas laws are being obeyed. "Thus when stated in terms of fugacities or activities the law of mass action has a universal validity" (La Mer, 1925). However, since many substances are non-volatile, it has been more convenient to replace the absolute activity by the relative activity, for which the standard state of reference is defined in terms either of the pure liquid or of the pure solid state instead of the attenuated gaseous state.

In accordance with these principles, we have the relationship

$$\gamma = \frac{\alpha}{c} \tag{1}$$

where c is the stoichiometric molal concentration, α is the activity concentration, and γ is the activity coefficient, which, in dilute solutions, may be regarded as the thermodynamic degree of dissociation. In the case of solutions, the standard state is conveniently taken so that at infinite dilution of the solute, γ becomes unity, and all the gas laws are obeyed. Hence α will be equal to c at infinite dilution. Although the fugacity and activity of a solute at infinite dilution are proportional by Henry's law to the mol fraction N, one may still use molality units c as proportional to N in dilute solutions. All of the data in these papers have been calculated on the molality basis; *i.e.*, in mols per kilo of H_2O.

Using the concept of activity, one may write for a strong electroyte such as NaCl,

$$\frac{\alpha_+ \times \alpha_-}{\alpha_0} = K \tag{2}$$

which is the mass law equation involving the thermodynamic concentrations or activities. The activity is related to the molal concentration so that

$$\alpha_+ = \gamma_+ c_+$$

$$\alpha_- = \gamma_- c_-$$

$$\alpha_0 = \gamma_0 c_0$$

$$\text{(3) therefore,} \qquad \frac{\gamma_+ c_+ \times \gamma_- c_-}{\gamma_0 c_0} = K$$

This is the thermodynamic mass law equation for dissociated electrolytes where subscripts +, −, and o denote positive ion, negative ion, and salt, respectively.

Lewis and Randall (1923), studying activity coefficients in mixtures of different valence types, made the generalization that "*in dilute solutions*, the activity coefficient of a given strong electrolyte is the same in all solutions of the same ionic strength." The ionic strength is related to the stoichiometric molality in the following manner:

Let μ = ionic strength
and $c_1\ c_2\ c_3 \ldots . c_n$ = molal concentration of ions present
$z_1\ z_2\ z_3 \ldots . z_n$ = valence of the ions,
then

$$\mu = \tfrac{1}{2}\ (c_1 z_1^2 + c_2 z_2^2 + c_3 z_3^2 \ldots . + c_n z_n^2) \tag{4}$$

Thus the concentration is related to the number and valence of ions present as they alter the electronic environment of a solution. It should be pointed out, however, that this is a limiting law, and serves only as a first approximation in solutions where individual ion properties are not significant.

The activity of a solute may be calculated from the vapor pressure of the solute, from the distribution of a solute between two solvents, from measurements of electromotive force, from freezing point data, and from solubility measurements. The solubility of slightly soluble salts in solutions of other salts has been utilized by many physical chemists, *e.g.*, Lewis, Brönsted, Noyes, Bray, Harkins, and their collaborators, to calculate the activity coefficients of the slightly soluble salts in the solution. It is this method which has been utilized in these studies.

At constant temperature, when a salt in solution is in equilibrium with the solid solute, it follows as a thermodynamic necessity that the activity of the salt in the solution is fixed and equal to the activity of the solid solute. No change in the solvent such as would be produced by addition of electrolytes or non-electrolytes can change that activity. If the activity coefficient of the salt in solution is decreased, due to a change in the solvent, more of the salt will go into solution until the activity in the solution and the solid state are equal. Hence

$$\gamma_o s_o = \gamma_1 s_1 = \gamma_2 s_2 \ldots . \tag{5}$$

where s_o refers to solubility in pure water, and s_1 and s_2 refer to solubility in other solvents.

$$\frac{\gamma_o}{\gamma_1} = \frac{s_1}{s_o} \qquad \text{or} \quad -\log \gamma_1 = p\gamma_1 = \log \frac{s_1}{s_o} - \log \gamma_o \tag{6}$$

Setting γ_o arbitrarily equal to unity,

$$-\log \gamma_1 = p\gamma_1 = \log \frac{s_1}{s_o} \tag{7}$$

If a salt A_{ν_1}, B_{ν_2} dissociates so that $A_{\nu_1}\ B_{\nu_2} \rightleftarrows \nu_1 A + \nu_2 B$ at equilibrium, the following relationship must hold true:

$$(\alpha A^+)^{\nu_1} \times (\alpha B^-)^{\nu_2} = K_{s.p.} \tag{8}$$

where αA^+ and αB^- denote the activities of the ions, ν_1 and ν_2 are the number of ions of A^+ and B^- respectively, + and − are used regardless of valence, and $K_{s.p.}$ is the activity product or solubility product at ionic strength equal to zero. Substituting γc for α, where γ, c, and α refer to the ions, equation (8) becomes

(9) $$(cA^+)^{\nu_1} \times (\gamma A^+)^{\nu_1} \times (cB^-)^{\nu_2} \times (\gamma B^-)^{\nu_2} = K_{s.p.}$$

or using the conventional symbol [ ] for concentration c,

(10) $$[A^+]^{\nu_1} (\gamma A^+)^{\nu_1} \times [B^-]^{\nu_2} (\gamma B^-)^{\nu_2} = K_{s.p.}$$

By definition, the activity coefficient of a salt is the geometrical mean of the activity coefficients of its ions. Hence

(11) $$\gamma A_{\nu_1} B_{\nu_2} = \sqrt[\nu_1 + \nu_2]{(\gamma A^+)^{\nu_1} \times (\gamma B^-)^{\nu_2}}$$

$$= (\gamma A^+)^{\frac{\nu_1}{\nu_1 + \nu_2}} \times (\gamma B^-)^{\frac{\nu_2}{\nu_1 + \nu_2}}$$

Logarithmically equation (11) becomes

(12) $$\log \gamma A_{\nu_1} B_{\nu_2} = \frac{\nu_1}{\nu_1 + \nu_2} \log \gamma A^+ + \frac{\nu_2}{\nu_1 + \nu_2} \log \gamma B^-$$

Equation (10) may be written in the form

(13) $$[A^+]^{\nu_1} \times [B^-]^{\nu_2} \times (\gamma A_{\nu_1} B_{\nu_2})^{\nu_1 + \nu_2} = K_{s.p.}$$

(14) $$[A^+]^{\nu_1} \times [B^-]^{\nu_2} = \frac{K_{s.p.}}{(\gamma A_{\nu_1} B_{\nu_2})^{\nu_1 + \nu_2}} = K'_{s.p.}$$

We have found it convenient to transform equation (14) into its logarithmic form

(15) $$\nu_1 \log [A^+] + \nu_2 \log [B^-] = \log K_{s.p.} - (\nu_1 + \nu_2) \log \gamma A_{\nu_1} B_{\nu_2} = \log K'_{s.p.}$$

As a further simplification, we have uniformly employed the symbol pX to designate $-\log [X^+]$. Equation (15) accordingly becomes

(16) $$\nu_1 \, pA^+ + \nu_2 \, pB^- = pK_{s.p.} - (\nu_1 + \nu_2) \, p\gamma A_{\nu_1} B_{\nu_2} = pK'_{s.p.}$$

(17) $$pK_{s.p.} - pK'_{s.p.} = (\nu_1 + \nu_2) \, p\gamma A_{\nu_1} B_{\nu_2} = \Delta \, pK_{s.p.}$$

(18) $$\text{or } p\gamma A_{\nu_1} B_{\nu_2} = \frac{\Delta pK_{s.p.}}{\nu_1 + \nu_2}$$

Equation (18) is thus identical with that derived by Brönsted and La Mer (1924) by substituting $\sqrt[\nu]{K_{s.p.}}$ for s in equations (5), (6), and (7).

Debye and Hückel (1923), from purely physical considerations, employing the theory of complete dissociation of strong electrolytes first advanced by Bjerrum (1909), have recently elaborated a theory which accounts for the behavior of these substances by taking into consideration the electrical forces at play between the ions. Their mathematical treatment has been further simplified and applied to salts of higher valence type by Brönsted and La Mer, who found that the activity coefficient was related to the ionic strength of the solution by the equation

(19) $$-\log \gamma_{salt} = \alpha' z_1 \, z_2 \sqrt{\mu}$$

where α' is a universal constant depending among other things on the dielectric constant of the medium, and z_1 and z_2 refer to the valences of the two ions of the salt. The addition of a correction term $\beta\mu$ enables one to use the equation for higher ionic strengths. More recently, Hückel (1925), developing the theory advanced by Milner and by Debye and Hückel, has derived a general working equation of the form

$$(20) \qquad -\log \gamma_{salt} = \frac{Bz_1z_2\sqrt{\mu}}{1 + A\sqrt{\mu}} + C\mu$$

In this equation A, B, and C denote constants. This equation takes into consideration the effect of the ionic environment, the size of the ions, and the dielectric properties of the solution. Since the concentrations of the solutions used in our experiments were less than 0.2 molal, we have neglected the possible deviation in the dielectric properties of the solutions with changing ionic strength. The equation used in these papers to relate the activity coefficient of the salts studied to the ionic strength has been of the general form

$$(21) \qquad -\log \gamma_{salt} = \frac{B\sqrt{\mu}}{1 + A\sqrt{\mu}}$$

For convenience in calculation, $Bz_1 z_2$ has been used as if it were one constant B.

These theoretical considerations will be further elaborated and discussed in connection with the experimental work as it is presented in later sections and papers. These sections given in order will contain a presentation of the technique used in the work, the calculations used, and the constants involved. Studies of the solubility of calcium carbonate and tertiary calcium phosphate, singly and together, under various conditions, will then follow.

Experimental Technique.

Analytical Methods.

Calcium.—Total calcium was determined by the method of Kramer and Tisdall (1921) slightly modified.[1] It was found that washing the precipitated and centrifuged calcium oxalate three times with 3 cc. of 2 per cent ammonia water, without stirring, gave results within 2 per cent of theoretical on solutions

[1] Some of the later determinations were checked by a gasometric method developed by Dr. Van Slyke, as yet unpublished. The washed precipitated calcium oxalate is dissolved in sulfuric acid as usual and transferred quantitatively into the Van Slyke manometric gas analyzer (Van Slyke and Neill, 1924). The oxalic acid is oxidized with an excess of permanganate under the proper conditions, and the yield of carbon dioxide, which is quantitative, is taken as a measure of the calcium in the precipitate.

of known calcium content. Titrations were made with N/100 or N/200 $KMnO_4$ using 1 to 5 cc. samples according to the calcium content of the solution analyzed. The permanganate factor was redetermined in every experiment by means of standard sodium oxalate.

Clark and Collip (1925) have demonstrated that the conditions necessary for obtaining theoretical results with this and similar methods are purely empirical. Kramer and Tisdall, later Tisdall (1923) and Clark and Collip have all endeavored to work under conditions such that the combined error due to solubility of calcium oxalate, and the retention of ammonium oxalate after washing, might be a minimum. As pointed out by the latter authors, any deviation in an arbitrary procedure will cause corresponding differences in results. Clark and Collip's modification, although quite satisfactory, was abandoned because of loss of precipitate when tubes were used which were not narrow enough at the bottom. In agreement with their findings, the original Kramer-Tisdall method gave low results. Some comparative results obtained by using different methods in determining the calcium content of a known solution are given below. Frequent determinations on the above solution, alone and when added to serum, served as a check on the accuracy of the method and the technique of the individual performing the analysis.

Determinations of Ca Content, by Different Methods, of an Aqueous Solution Containing 2.50 mM Ca per Liter.

Clark and Collip.	Kramer and Tisdall.	
	With stirring.	Without stirring.
2.48	2.32	2.50
2.48	2.32	2.51
2.46	2.32	2.51
2.53	2.35	2.52

Hydrogen Ion.—The $p\alpha_{H^+}$ of salt solutions and biological fluids was determined in the various ways indicated in the tables of experimental results: *e*, electrometric; *c*, colorimetric; *g*, gasometric results. Electrometric determinations were made in a thermostat maintained at 38 ± 1°. The temperature of the reaction liquid was read to 38 ± 0.2° in the Clark-Cullen electrode vessel of 2 cc. capacity. A thermometer in the electrode vessel, as suggested by Cullen, permitted accurate temperature control

of the liquid under investigation. The gas chain employed was the following.

Hg	HgCl	Saturated KCl	Saturated KCl bridge	Unknown solution	H_2	Pt

Pure mercury was prepared according to Hulett (1905) by redistilling five times. Calomel was prepared electrolytically from the purified mercury and redistilled hydrochloric acid. Merck's analytical KCl was used in the saturated calomel electrode and salt bridge. The hydrogen used was found by analysis to be 99.5 per cent pure. Whenever CO_2-containing solutions were being measured, the electrode was first flushed and filled with a mixture of hydrogen and CO_2, the latter gas being at the same tension as that of the CO_2 in the liquid. Usually the gas phase, with which the unknown had been equilibrated, was itself run from the large saturation tonometer into the electrode vessel, by displacement with mercury. Electrodes were made of heavy platinum foil. Before use in each experiment, they were freshly coated with platinum black. Platinic chloride for plating electrodes was prepared from pure platinum.

The standard used was N/10 HCl made from Hulett's (1909) constant boiling acid, twice redistilled. The strength of the N/10 HCl was confirmed by gravimetric analyses which indicated a chloride content within 0.1 per cent of theoretical. The $p\alpha_{H^+}$ of this acid, on the basis of Lewis' (1923) value (p. 382) for the activity of H^+ ion in a solution of this ionic strength, was assumed to be 1.08 at 38° and 20°. No correction was made for liquid junction potential.

Values of $p\alpha_{H^+}$ of unknown solutions were calculated from the equation:

$$p\alpha_{H^+} = \frac{E \text{ (corrected to 1 atmosphere of dry hydrogen)} - e_{o_2}}{0.0001983\ T}.$$

In many of the experiments, $p\alpha_{H^+}$ was determined colorimetrically on both salt solutions and serum, using bicolor standards

[2] It may be noted here, that $p\alpha_{H^+}$ referred to in these papers is identical with $p\alpha_{H^+}$ as used by Hastings and Sendroy (1925) and with the pH of Van Slyke, Hastings, Murray, and Sendroy (1925). However, this value is *not* identical with the $p\alpha_H$ of Sörensen and Linderström-Lang (1924), which is approximately 0.04 higher than ours, but corresponds to their pH, which is

and reading at 38°, according to the technique of Hastings and Sendroy (1924). From time to time, the color standards were checked against phosphate standards which in turn had been standardized electrometrically. Salt solutions were read *immediately* after sampling under oil for such solutions lose CO_2 and change their reaction very quickly. Colorimetric determinations on serum were accurate to $+0.05$ $p\alpha_{H^+}$, salt solutions to $+0.02$.[3]

Sometimes when a high degree of accuracy was required, or when the reaction was too acid or too alkaline to be read within the phenol red range, $p\alpha_{H^+}$ was indirectly determined from total CO_2 and CO_2 tension analyses, as outlined in the section on calculations.

CO_2 Tension.—The gas phase was analyzed in the Henderson-Haldane apparatus of 10 cc. capacity. The accuracy of these analyses was approximately 0.5 per cent.

CO_2 Content.—Total CO_2 was determined in the manometric gas apparatus described by Van Slyke and Neill (1924). These determinations were accurate to about 0.3 per cent.

H_2O Content.—Moisture determinations for converting data from molarity to molality basis were obtained by drying 1 or 2 cc. samples for about 15 to 20 hours in a Freas oven at 120°C. Weighings were made to 1 mg., and the error involved was not more than 0.2 per cent.

---

obtained by using the Bjerrum extrapolation for liquid junction potential, and assigning to N/10 HCl a value based on conductivity measurements. No correction for liquid junction potentials between saturated KCl and either N/10 HCl or our unknown solutions has been attempted. If in the future the difference between these diffusion potentials is established our $p\alpha_{H^+}$ values are subject to a corresponding correction.

[3] The bicolor standards referred to, as described previously (Hastings and Sendroy, 1924), have values based on the comparison of such standards against electrometrically standardized M/15 Sörensen phosphate standards. However, experiments as yet unpublished indicate that the effect of ionic strength and valence type of salt on the activity of dyes is similar to their effect on other ions. In other words, the apparent dissociation constant of the dye changes with ionic strength and with different salts in the solution, thus behaving in conformity with the Debye-Hückel theory of strong electrolytes. In order to detect any possible difference in activity of phenol red in the M/15 phosphate solution and in artificial edema fluid, such as was usually employed, comparative results were obtained by electrometric and colorimetric determinations of the same solutions. Since the two methods agreed in all cases to within 0.01 $p\alpha_{H^+}$, it was decided that all readings on balanced salt solutions obtained with the bicolor standards could be considered reliable.

Phosphorus.—Determinations of the phosphorus in salt solutions and serum were made according to the method of Benedict and Theis (1924), an improvement on the Briggs (1922) modification of the original Bell and Doisy (1920) colorimetric method. Stanford and Wheatley (1925) have recently furnished proof of the validity of the principle upon which the method depends, and report that there is a direct proportionality between the color produced and the phosphate present, only when the method is done under certain conditions. Inasmuch as Benedict and Theis also confirm Buell's (1923) finding that there is no appreciable amount of acid-soluble organic phosphorus in serum which is hydrolyzed by concentrated sulfuric acid, a determination by this method, even in serum, can most probably be taken to represent only the inorganic phosphorus, hence the total phosphate. In our experiments we have calculated our phosphate results on this assumption. We have confirmed Stanford and Wheatley in that accurate results can only be obtained when total acidity, concentration of all reagents, and temperature are strictly uniform in standards and unknown solutions.

The reading of the solution and standard must not differ by more than 20 per cent in order to obtain a reading which is within 5 per cent of theoretical. In serum analyses, the precipitation of proteins in a solution diluted only five times may very well lead to still greater error. However, this is unavoidable in such cases where the extremely low phosphate content of the solution analyzed makes it necessary to have as concentrated a filtrate as possible, in order to be able to read the slight coloration produced.

Saturation Procedure.

Salt solutions approximating quantitatively and qualitatively the content of edema fluid and other body fluids as analyzed by Salvesen (1923) were made up as indicated in the protocols. Standard solutions of NaCl, KCl, $MgCl_2$, $CaCl_2$, $NaHCO_3$, K_2SO_4, and Na_2HPO_4-KH_2PO_4 of known composition were prepared. Quantities of these solutions required to give the desired final concentration of each of these salts were accurately pipetted into a 200 cc. volumetric flask. Before the $CaCl_2$ was introduced, 125 cc. of water saturated with CO_2 were added to the flask to prevent premature precipitation of the calcium as carbonate or phosphate. The solution was then made up to 200 cc. and

transferred to the saturating tonometer system previously described by Austin and collaborators (1922). About 50 cc. of this salt solution were introduced into either a small tonometer or a 50 cc. narrow mouth centrifuge tube, about 1 per cent in weight of solid added, and then connection made with rubber tubing to a larger tonometer of from 300 to 400 cc. capacity.

The liquid with solid was clamped off from the large tonometer, which was washed out three times with either air or hydrogen, depending on whether or not $p\alpha_{H^+}$ was to be measured electrometrically; the tension of CO_2 required to give the desired final reaction was introduced, and then air or hydrogen run in to 1 atmosphere pressure. The liquid phase was then allowed to run into the large tonometer, the whole being placed in a water bath maintained at 38 $\pm$ 0.05° and rotated for 20 minutes. This procedure was repeated three more times, in order to eliminate excess CO_2 and so approach the desired final tension. At the fourth saturation, air or hydrogen was run in only to within 80 mm. of atmospheric pressure. The next day, after rotation from 18 to 24 hours, the whole was equilibrated to the atmosphere, then liquid and solid were separated from the gas phase. When saturation was carried on for several days, the CO_2 tension was adjusted several times during the saturation period, and then, finally, about 20 hours or so before being analyzed.

When the CO_2 tension was desired, samples of gas were taken in small 70 cc. Barcroft tubes or else sampled directly from the large tonometer. When centrifuge tubes were used for the liquid and solid, these were quickly placed in a centrifuge after covering with oil and rubber stoppers, or else paraffin, to prevent loss of CO_2. After centrifugation, the clear supernatant fluid was transferred to small containers over mercury. When small tonometers were used directly, the solid phase was removed by filtration through small glass tubes containing cotton.

Early in the work, when solutions were kept in contact with the solid salt, a constant temperature of 38° in centrifuging was maintained. However, it soon became apparent that the difference in solubility of calcium carbonate or phosphate between room and body temperature during the period between the end of saturation and completion of all analyses, was within the limit of error of the methods of analysis. When absorbent cotton was used for filtration, it was thought that stray shreds of cotton might affect the determination of the calcium. To test this,

comparisons were made on clear solutions, some containing a few shreds, and others more cotton, during the heating with normal sulfuric acid and subsequent titration. The presence of

TABLE I.

Effect of Variation within the Known Experimental Limit of Error of Any of the Factors in Calculation of $pK'_{s.p.}$ of $CaCO_3$ and $Ca_3(PO_4)_2$.

$p\alpha_{H^+}$	$[Ca^{++}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	Total $[CO_2]$ $\frac{M \times 10^3}{kg.\ H_2O}$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	pCa^{++}	$pCO_3^=$	$pPO_4^{\equiv}$	$pK'_{s.p.}$ $CaCO_3$	$pK'_{s.p.}$ $Ca_3(PO_4)_2$
7.43	0.28	30.32	0.293	3.55	3.90	7.95	7.45	26.55
7.38	0.28	30.32	0.293	3.55	3.95	8.01	7.50	26.67
−0.05							+0.05	+0.12
7.43	0.28	30.32	0.293	3.55	3.90	7.95	7.45	26.55
7.43	0.266	30.32	0.293	3.58	3.90	7.95	7.48	26.64
	−5 per cent.						+0.03	+0.09
7.43	0.28	30.32		3.55	3.90		7.45	
7.43	0.28	30.22		3.55	3.90		7.45	
		−0.3 per cent.					±0.00	
7.43	0.28		0.293	3.55		7.95		26.55
7.43	0.28		0.278	3.55		7.97		26.59
			−5 per cent.					+0.04
			Summary.					
±0.05							∓0.05	∓0.12
	±5 per cent.						∓0.03	∓0.09
		±0.3 per cent.					±0.00	
			±5 per cent.					∓0.04
Average error							±0.04	±0.08
Maximum "							±0.08	±0.25

cotton in these solutions within the time required to dissolve the calcium oxalate and to titrate, had no effect in reducing the permanganate.

The solid $CaCO_3$ and $Ca_3(PO_4)_2$ used were of the highest purity obtainable. Before use in these experiments, both were washed five times with distilled water, then dried at 110°.

Table I indicates the limit of accuracy attainable with these methods, and the effect of errors in determining the values $pK'_{s.p.}$ $CaCO_3$ and $pK'_{s.p.}$ $Ca_3(PO_4)_2$.

Symbols.

The symbols below have been used in this and the following papers.

$\alpha_{Ca^{++}}$ = activity of Ca^{++} ion.
$\gamma_{Ca^{++}}$ = " coefficient of Ca^{++} ion.
$[Ca^{++}]$ = concentration of Ca^{++} ion in mols per kilo of H_2O.
$[Ca]$ = " " total Ca " " " " " "
$[CaP]$ = " " Ca bound by protein in mols per kilo of H_2O.
$[CaX]$ = concentration of Ca not in ionized form in mols per kilo of H_2O.
μ = ionic strength.
ν = number of ions of a salt.
pCa^{++} = negative logarithm of $[Ca^{++}]$.
$p\gamma_{Ca^{++}}$ = " " " $\gamma_{Ca^{++}}$.
K = activity dissociation constant of an acid.
K′ = stoichiometric dissociation constant of an acid.
pK = negative logarithm of K.
$pK_{s.p.}$ $CaCO_3$ = $p[\alpha_{Ca^{++}} \times \alpha_{CO_3^{=}}]$.
$pK'_{s.p.}$ $CaCO_3$ = $p([Ca^{++}] \times [CO_3^{=}])$.
$\alpha^{\circ}_{H_2O}$ = solubility coefficient of CO_2 in pure water.
$\alpha^{\circ}_{CO_2}$ = " " " " " salt solution and serum.
pCO_2 = CO_2 tension in mm. of Hg.
f_3 = $pCO_2 : [CO_2]$.
f_2 = $[H_2CO_3] : [CO_2]$.
f_1 = $[HCO_3^-] : [CO_2]$.
f = $[CO_3^{=}] : [CO_2]$.
mM = millimols = M × 10^3.

Calculations.

Calculation of $[CO_3^{=}]$ Ion Concentration from Total CO_2 Content and $p\alpha_{H^+}$.

From the mass law,

$$\frac{\alpha_{H^+} \times \alpha_{HCO_3^-}}{\alpha_{H_2CO_3}} = K_1 \tag{22}$$

$$\frac{\alpha_{H^+} \times \gamma_{HCO_3^-} \times [HCO_3^-]}{\gamma_{H_2CO_3} \times [H_2CO_3]} = K_1 \tag{23}$$

(24) $$\frac{\alpha_{H^+} \times [HCO_3^-]}{\gamma_{H_2CO_3} \times [H_2CO_3]} = \frac{K_1}{\gamma_{HCO_3^-}} = K_1'$$

(25) $$\frac{\alpha_{H^+} \times [HCO_3^-]}{[H_2CO_3]} = \frac{K_1 \times \gamma_{H_2CO_3}}{\gamma_{HCO_3^-}} = K_1''$$

However, K_1'' is a *constant* only under conditions in which $\gamma_{H_2CO_3}$ and $\gamma_{HCO_3^-}$ are constant.

(26) In like manner $$\frac{\alpha_{H^+} \times [CO_3^=]}{[HCO_3^-]} = \frac{K_2 \times \gamma_{HCO_3^-}}{\gamma_{CO_3^=}} = K_2'$$

(27) $$\text{Total } [CO_2] = [H_2CO_3] + [HCO_3^-] + [CO_3^=]$$

By equation (26)

(28) $$[HCO_3^-] = \frac{\alpha_{H^+} \times [CO_3^=]}{K_2'}$$

By equations (25) and (28),

(29) $$[H_2CO_3] = \frac{\alpha_{H^+} \times [HCO_3^-]}{K_1''} = \frac{(\alpha_{H^+})^2 \times [CO_3^=]}{K_1'' K_2'}$$

Therefore, equation (27) can be written

(30) $$\text{Total } [CO_2] = \frac{(\alpha_{H^+})^2 \times [CO_3^=]}{K_1'' K_2'} + \frac{\alpha_{H^+} \times [CO_3^=]}{K_2'} + [CO_3^=]$$

(31) $$\text{Total } [CO_2] = [CO_3^=] \times \left[\frac{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2'}{K_1'' K_2'}\right]$$

(32) $$[CO_3^=] = \frac{\text{Total } [CO_2] \times K_1'' K_2'}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2'}$$

In a similar way

(33) $$[HCO_3^-] = \frac{\text{Total } [CO_2] \times \alpha_{H^+} K_1''}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2'}$$

Knowing K_1'', and K_2' for any particular solution, one may derive a set of values for each $p\alpha_{H^+}$, such that

(34) $$f = \frac{K_1'' K_2'}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2'}$$

and

(35) $$f_1 = \frac{\alpha_{H^+} K_1''}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2'}$$

TABLE II.

Factors for the Calculation of $[CO_3^{=}]$ and $[HCO_3^{-}]$ from the Total $[CO_2]$ Content in Salt Solutions of $\mu = 0.152$ and in Serum.

$pK_1'' = 6.13$, $pK_2' = 9.79$

$p\alpha_{H^+}$	f where $f \times [CO_2] = [CO_3^{=}]$	f_1 where $f_1 \times [CO_2] = [HCO_3^{-}]$	$p\alpha_{H^+}$	f where $f \times [CO_2] = [CO_3^{=}]$	f_1 where $f_1 \times [CO_2] = [HCO_3^{-}]$
6.0	0.00007	0.426	7.11	0.00189	0.904
6.1	0.00010	0.478	7.12	0.00194	0.906
6.2	0.00014	0.540	7.13	0.00199	0.908
6.3	0.00019	0.597	7.14	0.00204	0.909
6.4	0.00027	0.650	7.15	0.00209	0.911
6.5	0.00036	0.700	7.16	0.00214	0.913
6.6	0.00048	0.746	7.17	0.00219	0.914
6.7	0.00064	0.788	7.18	0.00224	0.916
6.80	0.00084	0.823	7.19	0.00230	0.918
6.81	0.00086	0.826	7.20	0.00236	0.919
6.82	0.00089	0.830	7.21	0.00242	0.921
6.83	0.00091	0.833	7.22	0.00248	0.923
6.84	0.00094	0.836	7.23	0.00254	0.924
6.85	0.00096	0.839	7.24	0.00260	0.926
6.86	0.00099	0.842	7.25	0.00266	0.927
6.87	0.00101	0.845	7.26	0.00273	0.928
6.88	0.00104	0.848	7.27	0.00280	0.930
6.89	0.00107	0.851	7.28	0.00287	0.932
6.90	0.00110	0.854	7.29	0.00294	0.933
6.91	0.00113	0.857	7.30	0.00301	0.934
6.92	0.00116	0.860	7.31	0.00309	0.936
6.93	0.00119	0.863	7.32	0.00317	0.937
6.94	0.00122	0.865	7.33	0.00325	0.938
6.95	0.00125	0.867	7.34	0.00333	0.939
6.96	0.00129	0.870	7.35	0.00341	0.940
6.97	0.00132	0.872	7.36	0.00349	0.941
6.98	0.00135	0.875	7.37	0.00358	0.942
6.99	0.00139	0.877	7.38	0.00367	0.943
7.00	0.00142	0.880	7.39	0.00376	0.944
7.01	0.00146	0.882	7.40	0.00385	0.945
7.02	0.00150	0.884	7.41	0.00394	0.946
7.03	0.00154	0.887	7.42	0.00404	0.947
7.04	0.00158	0.889	7.43	0.00414	0.948
7.05	0.00162	0.891	7.44	0.00424	0.949
7.06	0.00166	0.893	7.45	0.00434	0.950
7.07	0.00171	0.896	7.46	0.00444	0.951
7.08	0.00175	0.898	7.47	0.00455	0.952
7.09	0.00179	0.900	7.48	0.00466	0.953
7.10	0.00184	0.902			

TABLE II—*Concluded.*

$p\alpha_{H^+}$	f where $f \times [CO_2] = [CO_3^=]$	f_1 where $f_1 \times [CO_2] = [HCO_3^-]$	$p\alpha_{H^+}$	f where $f \times [CO_2] = [CO_3^=]$	f_1 where $f_1 \times [CO_2] = [HCO_3^-]$
7.49	0.00477	0.954	7.71	0.00802	0.967
7.50	0.00489	0.954	7.72	0.00821	0.967
7.51	0.00501	0.955	7.73	0.00841	0.968
7.52	0.00513	0.956	7.74	0.00861	0.968
7.53	0.00525	0.957	7.75	0.00881	0.968
7.54	0.00537	0.958	7.76	0.00901	0.969
7.55	0.00550	0.958	7.77	0.00922	0.969
7.56	0.00565	0.959	7.78	0.00943	0.969
7.57	0.00578	0.960	7.79	0.00965	0.970
7.58	0.00591	0.960	7.80	0.00988	0.970
7.59	0.00607	0.961	7.9	0.01249	0.9711
7.60	0.00621	0.961	8.0	0.01573	0.9712
7.61	0.00636	0.962	8.1	0.01979	0.9698
7.62	0.00651	0.963	8.2	0.02480	0.9669
7.63	0.00666	0.963	8.3	0.03112	0.9623
7.64	0.00681	0.964	8.4	0.03890	0.956
7.65	0.00697	0.964	8.5	0.04858	0.9474
7.66	0.00713	0.965	8.6	0.0604	0.9364
7.67	0.00730	0.965	8.7	0.0748	0.9227
7.68	0.00747	0.966	8.8	0.0921	0.9059
7.69	0.00765	0.966	8.9	0.1141	0.8844
7.70	0.00783	0.966	9.0	0.1394	0.8594

where f and f_1 are really factors representing the percentage of total $[CO_2]$ in the form of $[CO_3^=]$ and $[HCO_3^-]$ respectively. Values for use with salt solutions at $\mu = 0.152$ and with serum, between $p\alpha_{H^+}$ 6.0 and 9.0 at 0.1 intervals, and for $p\alpha_{H^+}$ between 6.8 and 7.8 at 0.01 intervals are given in Table II.

Calculation of $p\alpha_{H^+}$ from Analyses of CO_2 Content and CO_2 Tension.

In experiments in which it was not possible to determine $p\alpha_{H^+}$ directly, this value was obtained indirectly by calculation from analyses of the total CO_2 content and the CO_2 tension. Expressing H_2CO_3 in terms of the CO_2 tension, pCO_2,

$$(36) \quad \alpha_{H_2CO_3} = \frac{\alpha^o_{H_2O} \times pCO_2}{760 \times 0.0224} = \frac{0.557 \times pCO_2}{760 \times 0.0224} = 0.0327\ pCO_2$$

$$(37) \quad [H_2CO_3] = \frac{\alpha^o_{CO_2} \times pCO_2}{760 \times 0.0224} = \frac{(0.557 - 0.103\ \mu + 0.36\ P) \times pCO_2}{760 \times 0.0224}$$

where, according to the notation in unpublished work of Van Slyke, Hastings, and Neill, $\alpha^o_{H_2O}$ and $\alpha^o_{CO_2}$ are the solubility coefficients of CO_2 in pure

water, and in a salt- and protein-containing solution. μ has its usual significance and P indicates gm. of protein per gm. of H_2O in the equation. We can write equation (27) in the form

$$\text{Total } [CO_2] = f\,[CO_2] + f_1\,[CO_2] + f_2\,[CO_2] \tag{38}$$

where f, f_1, and f_2 refer to the percentage factor of the total $[CO_2]$ in the form of $[CO_3^{=}]$, $[HCO_3^{-}]$, and $[H_2CO_3]$ respectively. It has been shown how f and f_1 may be derived in equations (34) and (35). Hence f_2 may be found by difference, by

$$1.00 - f - f_1 = f_2 \tag{39}$$

By definition,

$$f_2\,[CO_2] = [H_2CO_3] = \frac{\alpha^{o}_{CO_2} \times pCO_2}{760 \times 0.0224} \tag{40}$$

For solutions of a *constant composition*, $\alpha^{o}_{CO_2}$ will be constant, and we may write

$$pCO_2 = \frac{760 \times 0.0224 \times f_2\,[CO_2]}{\alpha^{o}_{CO_2}} = f_3\,[CO_2] \tag{41}$$

where

$$f_3 = \frac{f_2 \times 760 \times 0.0224}{\alpha^{o}_{CO_2}} \tag{42}$$

By equations (39), (34), and (35)

$$\frac{K_1'' K_2' + \alpha_{H^+} K_1''}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2'} + f_2 = 1.00 \tag{43}$$

$$f_2 = 1 - \frac{K_1'' K_2' + \alpha_{H^+} K_1''}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2'} \tag{44}$$

$$f_2 = \frac{(\alpha_{H^+})^2}{(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2'} \tag{45}$$

$$\text{Hence, } f_3 = \frac{(\alpha_{H^+})^2 \times 760 \times 0.0224}{\alpha^{o}_{CO_2}\,[(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2']} \tag{46}$$

$$\text{and } pCO_2 = \frac{\text{Total } [CO_2] \times (\alpha_{H^+})^2 \times 760 \times 0.0224}{\alpha^{o}_{CO_2}\,[(\alpha_{H^+})^2 + \alpha_{H^+} K_1'' + K_1'' K_2']} \tag{47}$$

Equation (47) expresses the relationship between the CO_2 tension, total CO_2, and α_{H^+}. In actual practice, no attempt was made to calculate $p\alpha_{H^+}$ according to equation (47). Having calculated f and f_1 at various

$p\alpha_{H^+}$'s f_2 was found by difference, and then f_3, for the corresponding $p\alpha_{H^+}$ values. Then, by plotting f_3 against $p\alpha_{H^+}$ a curve was obtained for the re-

TABLE III.

Factors for the Calculation of $p\alpha_{H^+}$ from Total CO_2 Content and CO_2 Tension for Use in Serum.

$f_3 = \frac{pCO_2}{[CO_2]}$	$\overset{o}{\alpha}_{CO_2} = 0.565$
$p\alpha_{H^+}$	f_3
6.0	17.31
6.1	15.72
6.2	13.855
6.3	12.138
6.4	10.542
6.5	9.036
6.6	7.650
6.7	6.385
6.8	5.301
6.9	4.367
7.0	3.584
7.1	2.891
7.2	2.379
7.3	1.898
7.4	1.536
7.5	1.235
7.6	0.994
7.7	0.783
7.8	0.620
7.9	0.494
8.0	0.395
8.1	0.313
8.2	0.250
8.3	0.199
8.4	0.154
8.5	0.123
8.6	0.096
8.7	0.078
8.8	0.054
8.9	0.045
9.0	0.036

lationship $f_3 = \frac{pCO_2}{\text{total } [CO_2]}$ at any desired $p\alpha_{H^+}$. By determining the CO_2 tension and total CO_2, f_3 values obtained gave the corresponding $p\alpha_{H^+}$. Corresponding values of f_3 and $p\alpha_{H^+}$ are given in Table III.

Inasmuch as $\alpha^{o}_{CO_2}$ is a variable depending on the composition of a solution, the relationships given will not always hold for the factors f_2 and f_3 as given in the foregoing. In order to calculate $p\alpha_{H^+}$ from total $[CO_2]$ and CO_2 tension, in *solutions of changing composition*, one proceeds as follows, using the $\alpha^{o}_{CO_2}$ which is the *appropriate one for that solution* whose $p\alpha_{H^+}$ is to be determined. Van Slyke, Hastings, and Neill have found (unpublished results) that $\alpha^{o}_{CO_2}$ (cc. of CO_2 per gm. of H_2O) varies according to the empirical formula

$$\alpha^{o}_{CO_2} = 0.557 - 0.103\mu + 0.36P^{4} \tag{48}$$

where P = gm. of protein per gm. of H_2O.

The solutions for which $p\alpha_{H^+}$ was determined in the manner to be outlined here, were never at a reaction higher than about 7.5. Hence the $[CO_3^{=}]$ ion concentration was so small that it could be neglected. Equation (25) therefore could be written

$$\frac{\alpha_{H^+} \times ([CO_2] - [H_2CO_3])}{[H_2CO_3]} = K_1'' \tag{49}$$

By using the proper value for $\alpha^{o}_{CO_2}$ in the solution under investigation, $[H_2CO_3]$ was calculated according to equation (37), and then $p\alpha_{H^+}$ found by converting equation (49) into the well known logarithmic form of the Henderson-Hasselbalch equation:

$$p\alpha_{H^+} = pK_1'' + \log \frac{[CO_2] - [H_2CO_3]}{[H_2CO_3]} \tag{50}$$

We may derive the actual working equation corresponding to equation (47) as follows:

$$\gamma_{H_2CO_3} = \frac{\alpha_{H_2CO_3}}{[H_2CO_3]} \tag{51}$$

Substituting the values for $\alpha_{H_2CO_3}$ and $[H_2CO_3]$ given in equations (36) and (37)

---

[4] As this material goes to press, it is now evident that recent work gives as a more accurate expression for the solubility of CO_2 in salt and protein solutions, the following equation

$$\alpha^{o}_{CO_2} = 0.554 - 0.104\mu + 0.36\ P.$$

Our results would not be significantly altered by calculating according to the above equation.

(52) $$\gamma_{H_2CO_3} = \frac{0.557}{0.557 - 0.103\mu + 0.36P} = \frac{1}{1 - 0.185\mu + 0.65P}$$

(53) $$[H_2CO_3] = 0.0327\ pCO_2\ (1 - 0.185\mu + 0.65P)$$

Also by equation (36)

(54) $$p\alpha_{H_2CO_3} = 1.486 + p\ pCO_2$$

(55) $$p\alpha_{HCO_3^-} = p\gamma_{HCO_3^-} + p\ [HCO_3^-]$$

(56) $$p\gamma_{HCO_3^-} = 0.5\sqrt{\mu}$$

Combining equations (27), (53), (54), (55), (56),

(57) $$p\alpha_{HCO_3^-} = 0.5\sqrt{\mu} + p\{[CO_2] - [CO_3^=] - 0.0327\ pCO_2(1 - 0.185\mu + 0.65P)\}$$

But since $pK_1 = 6.33$ at 38°, equation (22) may be written in the logarithmic form

(58) $$p\alpha_{H^+} = 7.816 - 0.5\sqrt{\mu} + p\ p\ CO_2 - p\{[CO_2] - [CO_3^=] - 0.0327\ pCO_2 (1 - 0.185\mu + 0.65P)\}$$

In salt solutions containing no protein and in which the $[CO_3^=]$ concentration is negligible, equation (58) simplifies into

(59) $$p\alpha_{H^+} = 7.816 - 0.5\sqrt{\mu} + p\ p\ CO_2 - p\{[CO_2] - pCO_2\ (0.0327 - 0.00605\mu)\}$$

In serum of average salt and protein composition where $\mu = 0.16$ and $P = 0.07$ gm. per gm. of H_2O, equation (58) becomes

(60) $$p\alpha_{H^+} = 7.616 + p\ pCO_2 - p\{[CO_2] - 0.0332\ pCO_2\}$$

When the $[CO_3^=]$ is not neglible, *i.e.* when the $p\alpha_{H^+}$ is greater than 8.0, the $p\alpha_{H^+}$ is first approximated by equation (59) or (60), then the $[CO_3^=]$ is estimated by determining the value of f for the approximated $p\alpha_{H^+}$. The CO_2 content when multiplied by this value gives the $CO_3^=$ concentration. This value for $[CO_3^=]$ is then substituted in equation (58) and the $p\alpha_{H^+}$ again calculated. Since most $p\alpha_{H^+}$ values are less than 8.0, however, it is not often necessary to apply equation (58).

Constants Used in CO_2 Calculations.

The values of the first and second dissociation constants and activity coefficients used in the calculations were taken from the determinations of Hastings and Sendroy (1925). The equations given by them for these constants are

(61) $$pK_1' = 6.33 - 0.5\sqrt{\mu}$$

(62) $$pK_2' = 10.22 - 1.1\sqrt{\mu}$$

For the balanced salt solutions used, the values calculated for an approximate ionic strength of $\mu = 0.152$, were $pK_1' = 6.13$, $pK_1'' = 6.14$. Van Slyke, Hastings, Murray, and Sendroy (1925) found $pK_1' = 6.13$, $pK_1'' = 6.14$ for horse serum, while Hastings and Sendroy (unpublished results) have found $pK_1' = 6.12$ and $pK_1'' = 6.13$ in human serum. Therefore, in order to simplify the calculations, $pK_1'' = 6.13$ was used in experiments with the serum of horse and humans and in salt solutions of approximate $\mu = 0.152$. For the same ionic strength, $pK_2' = 9.79$. Hence $K_1'' = 7.41 \times 10^{-7}$ and $K_2' = 1.62 \times 10^{-10}$. For $\alpha^{\circ}_{H_2O}$ the value used was 0.557. In salt solutions and serum the variation of $\alpha^{\circ}_{CO_2}$ was calculated according to equation (48). These solubility coefficients were derived without attempting to differentiate between the two reactions.

$$\underset{\text{Gas.}}{CO_2} \rightleftarrows \underset{\text{Dissolved.}}{CO_2} \qquad \text{and} \qquad \underset{\text{Dissolved.}}{CO_2} + H_2O \rightleftarrows H_2CO_3$$

Reprinted from The Journal of Biological Chemistry
Vol. LXXI, No. 3, February, 1927

STUDIES OF THE SOLUBILITY OF CALCIUM SALTS.

II. THE SOLUBILITY OF TERTIARY CALCIUM PHOSPHATE IN SALT SOLUTIONS AND BIOLOGICAL FLUIDS.

By JULIUS SENDROY, Jr.,* and A. BAIRD HASTINGS.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

(Received for publication, September 2, 1926.)

Continuing the study of the solubility of the bone salts under various conditions, this paper has for its purpose the presentation of such studies in systems where solid $Ca_3(PO_4)_2$ was the sole saturating body. The general experimental analytical procedure outlined in Paper I was followed throughout. The presence of another important anion, namely $PO_4^{\equiv}$ ion, necessitated the use of another set of ionization constants and new tables of calculations. The necessary exposition of the material used in calculating these experiments is given in the following section. The calculations based upon CO_2 analyses have been given in Paper I.

Calculations.

Calculation of $PO_4^{\equiv}$ Ion Concentration from Analyses of Total $[PO_4]$ and the $p\alpha_{H^+}$.

In calculating their results, Holt, La Mer, and Chown (1925), did not have data available that permitted them to distinguish between the stoichiometric or apparent dissociation constants of phosphoric acid, K_1', K_2', and K_3' and the constants at zero ionic strength, or the activity dissociation constants, K_1, K_2, and K_3. In order to avoid this difficulty, and to obtain the proper values to use at different ionic strengths, an attempt was made to obtain, either from the literature or from experiments, some idea as to how these constants varied with ionic strength. The present section has for its purpose an explanation of how such constants were obtained

* This paper is part of a thesis submitted by Julius Sendroy, Jr., in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

and how they were used to calculate the concentrations of the three forms of phosphate in salt and serum calcium solubility product experiments.

Developing our equation in a manner similar to that adopted for calculating carbonate concentration, we have, from the mass law,

$$(1) \qquad \frac{\alpha_{H^+} \times \alpha_{H_2PO_4^-}}{\alpha_{H_3PO_4}} = K_1$$

$$(2) \qquad \frac{\alpha_{H^+} \times \gamma_{H_2PO_4^-} \times [H_2PO_4^-]}{\gamma_{H_3PO_4} \times [H_3PO_4]} = K_1$$

$$(3) \qquad \frac{\alpha_{H^+} \times [H_2PO_4^-]}{[H_3PO_4]} = \frac{K_1 \gamma_{H_3PO_4}}{\gamma_{H_2PO_4^-}} = K_1'$$

Similarly,

$$(4) \qquad \frac{\alpha_{H^+} \times [HPO_4^=]}{[H_2PO_4^-]} = \frac{K_2 \gamma_{H_2PO_4^-}}{\gamma_{HPO_4^=}} = K_2'$$

and

$$(5) \qquad \frac{\alpha_{H^+} \times [PO_4^{\equiv}]}{[HPO_4^=]} = \frac{K_3 \gamma_{HPO_4^=}}{\gamma_{PO_4^{\equiv}}} = K_3'$$

From equations (3), (4), and (5)

$$(6) \qquad [H_3PO_4] = \frac{(\alpha_{H^+})^3 \times [PO_4^{\equiv}]}{K_1' K_2' K_3'}$$

$$(7) \qquad [H_2PO_4^-] = \frac{(\alpha_{H^+})^2 \times [PO_4^{\equiv}]}{K_2' K_3'}$$

$$(8) \qquad [HPO_4^=] = \frac{\alpha_{H^+} \times [PO_4^{\equiv}]}{K_3'}$$

Hence, since

$$(9) \qquad \text{Total } [PO_4] = [H_3PO_4] + [H_2PO_4^-] + [HPO_4^=] + [PO_4^{\equiv}]$$

$$(10) \qquad \text{Total } [PO_4] = \frac{(\alpha_{H^+})^3 \times [PO_4^{\equiv}]}{K_1' K_2' K_3'} + \frac{(\alpha_{H^+})^2 \times [PO_4^{\equiv}]}{K_2' K_3'} + \frac{\alpha_{H^+} \times [PO_4^{\equiv}]}{K_3'} + [PO_4^{\equiv}]$$

Obtaining the concentrations of the mono-, di-, and trivalent phosphate ions in terms of the total phosphate, we have

$$(11)\quad [H_2PO_4^-] = \frac{\text{Total } [PO_4]\ (\alpha_{H^+})^2\ K_1'}{(\alpha_{H^+})^3 + (\alpha_{H^+})^2\ K_1' + \alpha_{H^+}\ K_1'\ K_2' + K_1'\ K_2'\ K_3'}$$

$$(12)\quad [HPO_4^=] = \frac{\text{Total } [PO_4]\ \alpha_{H^+}\ K_1'\ K_2'}{(\alpha_{H^+})^3 + (\alpha_{H^+})^2\ K_1' + \alpha_{H^+}\ K_1'\ K_2' + K_1'\ K_2'\ K_3'}$$

$$(13)\quad [PO_4^{\equiv}] = \frac{\text{Total } [PO_4]\ K_1'\ K_2'\ K_3'}{(\alpha_{H^+})^3 + (\alpha_{H^+})^2\ K_1' + \alpha_{H^+}\ K_1'\ K_2' + K_1'\ K_2'\ K_3'}$$

For the calculations in this work, the magnitudes of the first and fourth terms of the denominator, $(\alpha_{H^+})^3$ and $K_1'\ K_2'\ K_3'$, are such that they may be neglected.

Thus equations (11), (12), and (13) may be simplified:

$$(14)\quad [H_2PO_4^-] = \frac{\text{Total } [PO_4]\ (\alpha_{H^+})^2\ K_1'}{(\alpha_{H^+})^2\ K_1' + \alpha_{H^+}\ K_1'\ K_2'}$$

$$(15)\quad [HPO_4^=] = \frac{\text{Total } [PO_4]\ \alpha_{H^+}\ K_1'\ K_2'}{(\alpha_{H^+})^2\ K_1' + \alpha_{H^+}\ K_1'\ K_2'}$$

$$(16)\quad [PO_4^{\equiv}] = \frac{\text{Total } [PO_4]\ K_1'\ K_2'\ K_3'}{(\alpha_{H^+})^2\ K_1' + \alpha_{H^+}\ K_1'\ K_2'}$$

When $[PO_4^{\equiv}]$ and $[HPO_4^=]$ are known, of course, $[H_2PO_4^-]$ is found from total $[PO_4]$ by difference, instead of by the laborious use of equation (14).

First Dissociation Constant of Phosphoric Acid.

From equation (3) we obtain, on taking the negative logarithm of all terms,

$$(17)\quad pK_1' = pK_1 + p\gamma_{H_3PO_4} - p\gamma_{H_2PO_4^-}$$

Since we have no data on the activity coefficient of H_3PO_4, it is assumed to be unity. Therefore, $p\gamma_{H_3PO_4}$ disappears, and we have

$$(18)\quad pK_1' = pK_1 - p\gamma_{H_2PO_4^-}$$

For the activity coefficient of an ion, in dilute solutions, Brönsted and La Mer (1924) have given the limiting equation developed as a consequence of the Debye-Hückel theory,

$$(19) \qquad -\log \gamma_{ion} = 0.5\, z_i^2 \sqrt{\mu}$$

where z_i is the valence of the ion. Hence, theoretically, in dilute solutions, we should have $p\gamma_{H_2PO_4^-} = 0.5\sqrt{\mu}$, and $pK_1' = pK_1 - 0.5\sqrt{\mu}$.

Examination of the literature revealed that very little work had been done on this constant. Noyes and Eastman (1907), by conductivity measurements, determined the effect of varying concentrations of phosphoric acid on the first dissociation constant

TABLE I.

First Dissociation Constant of Phosphoric Acid at 18°.

Recalculated from Noyes and Eastman's data given by Abbott and Bray (p. 749).

Formula weights per liter.	$K_1' \times 10^2$.	Corrected $K_1' \times 10^2$.	pK_1'	Approximate $\sqrt{\mu}$.
0.1	1.12	1.12	1.95	0.316
0.08	1.07	1.07	1.97	0.283
0.05	1.01	1.01	1.995	0.224
0.0125	0.90	0.90	2.045	0.112
0.01	0.88	0.88	2.055	0.100
0.002	0.71	0.81	2.09	0.045
0.0002	0.63	0.65	(2.185)*	0.014

* Not used in plotting the data.

at 18°, and their results were found recalculated in the work of Abbott and Bray (1909) (p. 749, Table X). Table I contains these values corrected for one or two errors, apparently of calculation. The ionic strength has not been calculated on the molality basis, but this is unnecessary here, inasmuch as the calculations are subject to much greater errors. The temperature is 20° lower than that for which we have adopted these figures, but the temperature coefficient of such weak acids in this range is probably not very great. Furthermore, the concentration was varied by increasing concentrations of H_3PO_4. There can be no doubt that the specific ion effect in such a solution may be quite different from that in one of NaCl of the same concentration.

However, although the values calculated by it may be greatly in error, we have adopted for our calculation the equation which seems best fitted to suit the data, as affording some indication of the variation in magnitude of this constant between infinite dilution and the salt concentrations used in these experiments.

The data have been plotted in Fig. 1 with pK_1' as ordinates and $\sqrt{\mu}$ as abscissæ. Extrapolation to $\sqrt{\mu} = 0$ gives the empirical equation which fits the data, as

(20) $$pK_1' = 2.11 - 0.5\sqrt{\mu}$$

(21) $$\text{Hence, } p\gamma_{H_2PO_4^-} = 0.5\sqrt{\mu}$$

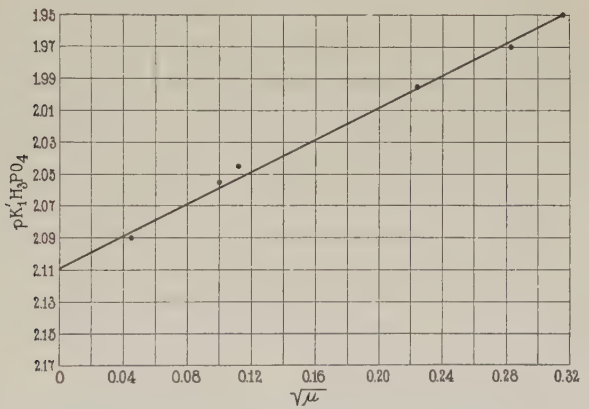


FIG. 1. The variation in the first dissociation constant of H_3PO_4 with ionic strength. Values of pK_1' are plotted as ordinates and $\sqrt{\mu}$ as abscissæ. The equation of the solid line is $pK_1' = 2.11 - 0.5\sqrt{\mu}$.

Abbott and Bray give $K_1' = 1.1 \times 10^{-2}$ at a normality of about 0.09, or $\sqrt{\mu} = 0.3$. Accordingly, using $p\gamma_{H_2PO_4^-} = 0.5\sqrt{\mu}$, pK_1 would be 2.12 which is in good agreement with Noyes and Eastman's data. For salt solutions at $\sqrt{\mu} = 0.390$ and for serum, we have used the value $K_1' = 1.22 \times 10^{-2}$ or $pK_1' = 1.915$.

Second Dissociation Constant of Phosphoric Acid.

From equation (4) we obtain

(22) $$pK_2' = pK_2 + p\gamma_{H_2PO_4^-} - p\gamma_{HPO_4^=}$$

The Brönsted-La Mer equation given above would make $p\gamma_{HPO_4^=} = 2.0\sqrt{\mu}$. Hence in dilute solutions, pK_2' should = $pK_2 - 1.5\sqrt{\mu}$.

For this constant various authors have given values in the literature. Apparently the stoichiometric dissociation constant pK_2' is greatly affected by the medium in which it is determined. Unpublished experiments done in this laboratory give as a first approximation, pK_2' at 20° = $7.18 - 1.25\sqrt{\mu}$ where the ionic strength was varied by increasing concentrations of NaCl. The pK_2' was determined by the equation

$$p\alpha_{H^+} = pK_2' + \log \frac{[HPO_4^=]}{[H_2PO_4^-]} \tag{23}$$

Varying proportions of the monobasic and dibasic phosphate were mixed and $p\alpha_{H^+}$ determined electrometrically. Assuming that the temperature coefficient of 0.03 obtained by Hastings and Sendroy (1924) holds for all dilutions,

$$pK_2' \text{ at } 38° = 7.15 - 1.25\sqrt{\mu} \tag{24}$$

and

$$p\gamma_{HPO_4^=} = 1.75\sqrt{\mu} \tag{25}$$

We have used values for pK_2' based on these formulas in all of our phosphate calculations.

Michaelis and Kruger (1921) and Michaelis and Garmendia (1914) have also published results with which our data agree very well. Their values for pK_2' in solutions of varying sodium chloride, phosphate, and sulfate content, indicate a difference in activity coefficient of the dibasic phosphate ion due to specific ion effects. However, inasmuch as our experimental salt solutions and serum contain a predominance of NaCl over the concentration of the other salts present, we have taken our value for pK_2' at different ionic strengths as determined by the results with solutions of NaCl, and have used the constants so obtained in calculating all phosphate determinations.

Abbott and Bray (p. 755, Table XVII) give a value, when recalculated, of $pK_2' = 6.73$ at 38° for $\sqrt{\mu} = 0.55$ in a solution of sodium ammonium phosphate salts. This value fits in with the data cited before and also with our own.

For salt solutions at $\sqrt{\mu} = 0.390$ and for serum, we have used the value $K_2' = 2.19 \times 10^{-7}$ or $pK_2' = 6.66$.

Third Dissociation Constant of Phosphoric Acid.

From equation (5) we obtain

$$pK_3' = pK_3 + p\gamma_{HPO_4^=} - p\gamma_{PO_4^{\equiv}} \tag{26}$$

The Brönsted-La Mer equation would make $p\gamma_{PO_4^{\equiv}} = 4.5\sqrt{\mu}$, and $pK_3' = pK_3 - 2.5\sqrt{\mu}$. For this constant, Abbott and Bray, Blanc (1920), Brydges (1911), and Prideaux and Ward (1924) have given different values ranging from 3.6×10^{-13} to 1×10^{-12}. Most of these results were obtained at room temperature and under various experimental conditions. However, no information could be found upon the variation of the third stoichiometrical dissociation constant with varying ionic strength. Accordingly, in order to obtain some idea of the effect of varying concentration on the activity of $PO_4^{\equiv}$ ion, an attempt was made to obtain the necessary values at 38° experimentally. This was done by determining the $p\alpha_{H^+}$ and the concentration of tribasic and dibasic phosphate ions in solutions whose ionic strength was varied by NaCl. Since it was impossible to work in CO_2-free solutions, the CO_2 content was also determined and correction made for the amount of Na_2CO_3 and $NaHCO_3$ present; correction was also made for the concentration of NaOH. These results have been plotted in Fig. 2 with pK_3' as ordinates and $\sqrt{\mu}$ as abscissæ. The empirical equation approximately fitting these data is

$$pK_3' = 12.66 - 2.25\sqrt{\mu} \tag{27}$$

from which

$$p\gamma_{PO_4^{\equiv}} = 4.0\sqrt{\mu} \tag{28}$$

Although the points determining the line which fits the above equation lie between concentrations $\sqrt{\mu} = 0.25$ and 0.50, and it is realized that the extrapolation to zero ionic strength involves an error of considerable magnitude, in the absence of better data we have been forced to use the above value for all phosphate calculations. Most of the experiments on salt solutions, and certainly those on serum, have concentrations between the above

limits. For salt solutions at $\sqrt{\mu} = 0.390$ and for serum we have used the value $K_3' = 1.66 \times 10^{-12}$ or $pK_3' = 11.78$.

It is believed, that while the three constants for phosphoric acid derived in this section may be quite in error, particularly K_1 and K_3, nevertheless, they seem the best available at the present time.

EXPERIMENTAL RESULTS.

Solubility of $Ca_3(PO_4)_2$ in Salt Solutions.—In view of the thorough study made by Holt, La Mer, and Chown, we have made no attempt to study aqueous systems in equilibrium with $Ca_3(PO_4)_2$, as completely as those in equilibrium with $CaCO_3$ and with both

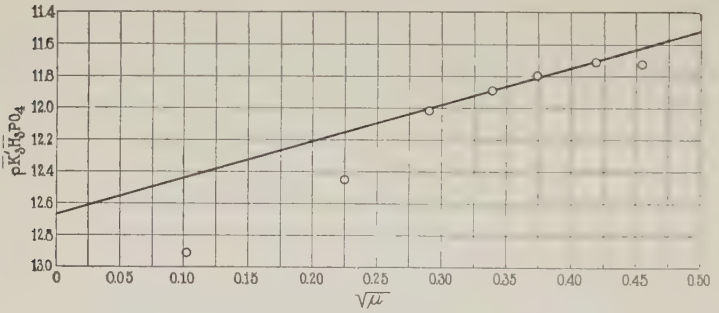


FIG. 2. The variation in the third dissociation constant of H_3PO_4 with ionic strength. Values of pK_3' are plotted as ordinates and $\sqrt{\mu}$ as abscissæ. The equation of the solid line is $pK_3' = 12.66 - 2.25\sqrt{\mu}$.

salts. In order that our picture of the various systems might be complete, however, and the results comparable with each other, a few experiments in which only solid $Ca_3(PO_4)_2$ was the solid phase have been performed.

Holt, La Mer, and Chown studied the solubility of $Ca_3(PO_4)_2$ in salt solutions of varying ionic strength. Using the constants for phosphoric acid which we have in part determined and in part obtained from the literature, we have recalculated their data in order that our experimental results may be compared with theirs. These experiments are discussed in Paper III in connection with the variation in solubility product of $Ca_3(PO_4)_2$ with ionic strength.

The aqueous systems in which $pK'_{s.p.}$ $Ca_3(PO_4)_2$ was determined all contained salts in amount and kind comparable to those found

in serum and transudates. The solutions were equilibrated at 38° from 20 hours to 8 days with solid $Ca_3(PO_4)_2$ at definite CO_2 tensions. At the end of the saturation period, the liquid phase was analyzed for CO_2, total phosphate, calcium, and $p\alpha_{H^+}$. From these results were calculated $[CO_3^=]$ and $[PO_4^{\equiv}]$ concentrations. Each of these, when multiplied by the calcium ion concentration, is the apparent solubility product for the salt studied. The results are given in Table II. In all of these experiments the

TABLE II.

Solubility of $Ca_3(PO_4)_2$ in Salt Solutions Saturated at 38° with Solid $Ca_3(PO_4)_2$.

Experiment No.	Saturation time.	Initial concentrations.			Final concentrations.		
		$[HCO_3^-]$ M × 10^3 / kg. H_2O	Total $[PO_4]$ M × 10^3 / kg. H_2O	$[Ca^{++}]$ M × 10^3 / kg. H_2O	Total $[CO_2]$ M × 10^3 / kg. H_2O	$[HCO_3^-]$ M × 10^3 / kg. H_2O	$[CO_3^=]$ M × 10^3 / kg. H_2O
7	20 hrs.	30.27	0.0	0.0	27.14	25.58	0.097
10	8 days.	30.27	0.0	0.0	28.53	27.17	0.130
6	20 hrs.	30.27	0.0	7.76	18.17	16.64	0.041
4	20 "	30.27	1.21	0.0	25.66	24.19	0.094
3	20 "	35.32	1.21	4.65	25.05	23.48	0.079

	Final concentrations.						
	Total $[PO_4]$ M × 10^3 / kg. H_2O	$[PO_4^{\equiv}]$ M × 10^3 / kg. H_2O	$[Ca^{++}]$ M × 10^3 / kg. H_2O	$p\alpha_{H^+}$	$pK'_{s.p.}$ $CaCO_3$	$pK'_{s.p.}$ $Ca_3(PO_4)_2$	$\sqrt{\mu}$
7	1.97	6.42	0.05	7.37	8.31	27.28	0.381
10	0.96	4.07	0.03	7.47	8.41	28.34	0.385
6	0.126	0.242	0.59	7.18	7.62	26.93	0.376
4	3.69	12.36	0.04	7.38	8.43	27.00	0.379
3	2.03	5.79	0.04	7.32	8.50	27.68	0.384

final concentration of calcium was so low that the results are not as reliable as those of our other experiments. However, it is quite apparent that in Experiments 4, 7, and 10, in which no calcium was originally present, the amount of calcium in solution at equilibrium was not sufficient to exceed the solubility product of $CaCO_3$. The values of $pK'_{s.p.}$ $Ca_3(PO_4)_2$, however, are more nearly of the order of magnitude to be expected in solutions of this ionic strength.

Experiment 4 is of particular interest because although the initial phosphate concentration was 1.21 mM, the final concentration was 3.69, indicating that 1.24 mM $Ca_3(PO_4)_2$ had gone into solution. Had none of the calcium precipitated, there would have been 3.72 mM calcium present at the end of the experiment, whereas analysis showed only 0.04 mM. If this calcium had precipitated as $CaCO_3$, there would have been a fall of 7.36 mM in $[HCO_3^-]$. The fall was actually 6.11 mM. This suggests that possibly some of the $Ca_3(PO_4)_2$ reacted with NaH_2PO_4 in the solution with the resulting precipitation of $CaHPO_4$.

In Experiments 6 and 3, calcium was initially present in solution; phosphate was initially present in solution in Experiment 3, but not in No. 6. After 20 hours saturation, most of the calcium had been precipitated and 0.126 mM of phosphate had been dissolved. The product $pK'_{s.p.}$ $Ca_3(PO_4)_2$ was nearly normal but $pK'_{s.p.}$ $CaCO_3$ was high, indicating unsaturation.

All of the values for $pK'_{s.p.}$ $Ca_3(PO_4)_2$ were higher than the corresponding values in salt solutions saturated with both solids. In the main, these experiments illustrate the greater difficulty which is encountered in bringing to equilibrium a system involving $Ca_3(PO_4)_2$, particularly if one starts from zero calcium concentration. Under these conditions, the solubility product of $CaCO_3$ was not equalled or exceeded.

These results are in marked contrast to similar experiments in which only $CaCO_3$ was the saturating body. Under such conditions, saturation of the solution with $CaCO_3$ resulted in apparent *supersaturation* in the case of *both* $CaCO_3$ and $Ca_3(PO_4)_2$ when phosphate was initially present in the solution, regardless of the initial calcium content. Here, saturation with solid $Ca_3(PO_4)_2$ for as long as 8 days did not result in the solubility product of $CaCO_3$ being equalled. Furthermore, even when an excess of calcium was initially present, the solution was still unsaturated with respect to $CaCO_3$. Inasmuch as the solutions of this section were not equilibrated in the presence of solid $CaCO_3$, results obtained for $pK'_{s.p.}$ were not contrary to anything that might have been expected for such systems.

Solubility of $Ca_3(PO_4)_2$ in Serum.—From analyses of the Ca, inorganic phosphate, and $p\alpha_{H^+}$ of serum as drawn, one may

calculate the product $[Ca]^3 \times [PO_4^{\equiv}]^2$.* In horse serum at $p\alpha_{H^+} = 7.40$ this is approximately 22.50 when expressed as $p([Ca]^3 \times [PO_4^{\equiv}]^2)$ and agrees well with values found by Holt, La Mer, and Chown when recalculated with the use of the constants given in the previous section (see Table III). This represents a high degree of apparent supersaturation over the value found at equilibrium in salt solutions of the same ionic strength.

TABLE III.

$p([Ca]^3 \times [PO_4^{\equiv}]^2)$ of Serum before and after Shaking with $Ca_3(PO_4)_2$ at 38°.

Recalculated data of Holt, La Mer, and Chown (p. 575).

$p\alpha_{H^+}$	[Ca] $\frac{M \times 10^3}{kg.\ H_2O}$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[PO_4^{\equiv}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$pPO_4^{\equiv}$	pCa	$pCa_3(PO_4)_2$
I. Human T. C. G. H_2O assumed, 930 gm. per liter.						
7.40	2.72	1.11	3.92	7.407	2.565	22.51
7.70	0.484	0.520	3.96	7.402	3.349	24.84
II. Human C. O'D. H_2O assumed, 930 gm. per liter.						
7.40	2.74	1.22	4.31	7.365	2.562	22.42
7.70	0.672	0.503	3.83	7.417	3.298	24.73
III. Horse 5515-H. H_2O assumed, 940 gm. per liter.						
7.35	2.93	1.17	3.61	7.443	2.533	22.49
7.40	0.718	0.720	2.54	7.595	3.144	24.62

Thus $Ca_3(PO_4)_2$ as in the case of $CaCO_3$, is present in serum as drawn in a condition of apparent supersaturation. Unlike the behavior of serum in the presence of $CaCO_3$, however, saturation with $Ca_3(PO_4)_2$ results in the precipitation of calcium. Holt, La Mer, and Chown showed this to be the case for human and horse serum by analyses of the serum before and after saturation

* Since $pK'_{s.p.}\ Ca_3(PO_4)_2 = 3\ pCa^{++} + 2\ pPO_4^{\equiv}$, it is erroneous to use $pK'_{s.p.}\ Ca_3(PO_4)_2$ for the term $3\ pCa + 2\ pPO_4^{\equiv}$. Here Ca denotes the *total* calcium, and until it can be definitely proved that this quantity is all ionized calcium, the use of $pK'_{s.p.}\ Ca_3(PO_4)_2$ should be carefully restricted. For convenience, we shall hereafter refer to $p([Ca]^3 \times [PO_4^{\equiv}]^2)$ as p $Ca_3(PO_4)_2$ and $p([Ca] \times [CO_3^{=}])$ as $pCaCO_3$.

with $Ca_3(PO_4)_2$ for a period of 1 to 2 weeks. In their three experiments (Table III), the calcium decreased 2.24, 2.08, 2.21 mM per kilo of H_2O; correspondingly, the total inorganic phosphate decreased 0.59, 0.72, 0.45 mM. These data show that the calcium could not have been precipitated entirely as $CaHPO_4$ or even as $Ca_3(PO_4)_2$ which has the highest Ca:P ratio. Calculated with our constants, the value of $p([Ca]^3 \times [PO_4^{\equiv}]^2)$ or $pCa_3(PO_4)_2$ = 24.84, 24.73, and 24.62, respectively, after saturation as compared with the value 22.50 before saturation.

We have confirmed this result in the following manner. Horse serum was equilibrated with $Ca_3(PO_4)_2$ at a tension designed to keep the $p\alpha_H$ approximately 7.4. After varying intervals of time, samples were removed for analysis of the CO_2, inorganic

TABLE IV.

Effect of Saturating Horse Serum at 38° with Solid $Ca_3(PO_4)_2$ for Varying Lengths of Time.

Experiment No.	Saturation time.	$p\alpha_{H^+}$ (colorimetric).	Total $[CO_2]$ M × 10^3 / kg. H_2O	$[HCO_3^-]$ M × 10^3 / kg. H_2O	$[CO_3^=]$ M × 10^3 / kg. H_2O	Total $[PO_4]$ M × 10^3 / kg. H_2O	$[PO_4^{\equiv}]$ M × 10^3 / kg. H_2O	[Ca] M × 10^3 / kg. H_2O	$pCaCO_3$	$pCa_3(PO_4)_2$
1	0	7.47	32.32	30.78	0.147	0.787	3.33	3.19	6.33	22.46
2	5 min.	7.28	28.91	26.96	0.083	0.904	2.31	0.74	7.21	24.66
3	1 hr.	7.37	27.13	25.57	0.097	0.815	2.66	0.62	7.22	24.78
4	3½ hrs.	7.37	27.39	25.80	0.098	0.860	2.80	0.62	7.23	24.77

phosphate, Ca, and $p\alpha_{H^+}$. The results of this experiment are given in Table IV. It is seen that the greatest precipitation of calcium took place within the first 5 minutes and was practically complete at the end of 1 hour. Longer saturation failed to bring about further precipitation of calcium.

The form in which it precipitated under these conditions is a matter of great interest. As there was little or no change in the inorganic phosphate during the experiment, the possibility of the calcium having been precipitated as a phosphate seems precluded. There was, however, a marked decrease in the bicarbonate concentration which quantitatively accounts for the precipitation of the calcium as $CaCO_3$.

In the first interval the decrease in calcium was 2.45 mM. If it precipitated as $CaCO_3$, the $[HCO_3^-]$ concentration would have

fallen 4.90. Allowing for the change in $p\alpha_{H^+}$, the decrease in $[HCO_3^-]$ was 4.66 mM. Similarly, in the next interval one may calculate from the decrease in calcium, an expected fall of 0.24 in $[HCO_3^-]$. By analysis the decrease was 0.35 mM. It would seem then, that one cannot be dealing here with a system supersaturated with respect to $Ca_3(PO_4)_2$ in the usual sense of the term.

This result is quite comparable to that obtained with the sodium citrate solutions mentioned in Paper I. There, the citrate solution was first brought into equilibrium with $CaCO_3$. Upon exchanging the $CaCO_3$ for $Ca_3(PO_4)_2$ as the saturating body, calcium carbonate was precipitated. This illustrates a behavior of citrate solutions toward calcium salts, analogous to that of serum. The significance of these results will be discussed in the following paper.

SUMMARY.

1. From data in the literature, the equation relating the first dissociation constant of phosphoric acid to the ionic strength of the solution has been found to be $pK_1' = 2.11 - 0.5\sqrt{\mu}$ at 18°. This value was applied to our work done at 38°.

2. From our own data, and from that in the literature, the equation relating the second dissociation constant of phosphoric acid to the ionic strength of the solution has been found to be $pK_2' = 7.15 - 1.25\sqrt{\mu}$ at 38°.

3. From our own data, the equation relating the third dissociation constant of phosphoric acid to the ionic strength has been found to be $pK_3' = 12.66 - 2.25\sqrt{\mu}$ at 38°.

4. The solubility of $Ca_3(PO_4)_2$ in salt solutions under various conditions has been studied.

5. The solubility of $Ca_3(PO_4)_2$ in serum has been studied. The results indicate a rapid precipitation of $CaCO_3$ when solid $Ca_3(PO_4)_2$ is added to serum.

Reprinted from The Journal of Biological Chemistry
Vol. LXXI, No. 3, February, 1927

STUDIES OF THE SOLUBILITY OF CALCIUM SALTS.

III. THE SOLUBILITY OF CALCIUM CARBONATE AND TERTIARY CALCIUM PHOSPHATE UNDER VARIOUS CONDITIONS.

By JULIUS SENDROY, Jr.,* and A. BAIRD HASTINGS.

(From the Hospital of The Rockefeller Institute for Medical Research, New York.)

(Received for publication, September 2, 1926.)

It is well known that the two primary bone salts are $CaCO_3$ and $Ca_3(PO_4)_2$, with more or less $CaHPO_4$. Probably the best available figures for the amount of $CaCO_3$ in bone are those of Goto (1918) whose analyses for calcium and for carbonate by an improved method of Van Slyke (1918) give values in bone of normal rabbits of 14.3 per cent calcium and 5.0 per cent CO_2 (carbonate 6.8 per cent). The figures for phosphate cannot be used because they include the organic phosphate of the marrow lipoids. Calcium carbonate would evidently be 11.3 per cent, and tertiary calcium phosphate, if all of the remaining calcium be assumed in that form, would be 25.3 per cent, giving a $CaCO_3$: $Ca_3(PO_4)_2$ ratio of 31 per cent : 69 per cent. Although some carbonate may have been present as the sodium salt, this is nevertheless a decidedly higher figure than the 15 : 85 per cent ratio commonly quoted for the bone salts, and places somewhat more stress on the importance of calcium carbonate in calcium metabolism.

In the absence of evidence as to the presence of $CaHPO_4$ in bone, the study of the solubility of the calcium salts of bone was confined to $CaCO_3$ and $Ca_3(PO_4)_2$. Having obtained the results of the preceding paper for tricalcium phosphate under various conditions, and in view of the results of Holt, La Mer, and Chown (1925) which appeared while the study of calcium carbonate

* This paper is part of a thesis submitted by Julius Sendroy, Jr., in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

equilibria was in progress, it seemed desirable, since bone contains solid $CaCO_3$ and $Ca_3(PO_4)_2$, to ascertain the equilibrium conditions for salt solutions and serum in the presence of these two solids. *A priori*, one would expect that equilibrium for both, that is a condition of saturation for both salts, would only be obtained in the presence of the two salts as saturating bodies. The experiments performed clearly indicate, with a few exceptions that seem fairly well capable of explanation, that such is the case.

In accordance with the theoretical considerations given, for any salt solution in equilibrium with solid $CaCO_3$ and $Ca_3(PO_4)_2$, the following equations hold:

(1) $$\alpha_{Ca^{++}} \times \alpha_{CO_3^{=}} = K_{s.p.\ CaCO_3}$$

(2) $$(\alpha_{Ca^{++}})^3 \times (\alpha_{PO_4^{\equiv}})^2 = K_{s.p.\ Ca_3(PO_4)_2}$$

Eliminating $\alpha_{Ca^{++}}$, one obtains

(3) $$\frac{\alpha_{CO_3^{=}}}{(\alpha_{PO_4^{\equiv}})^{\frac{2}{3}}} = \frac{K_{s.p.\ CaCO_3}}{K_{s.p.\ (Ca_3(PO_4)_2)^{\frac{1}{3}}}}$$

For any solution of fixed ionic strength,

(4) $$\frac{[CO_3^{=}]}{[PO_4^{\equiv}]^{\frac{2}{3}}} = \frac{K'_{s.p.\ CaCO_3}}{K'_{s.p.\ (Ca_3(PO_4)_2)^{\frac{1}{3}}}}$$

or expressed in terms of negative logarithms

(5) $$pCO_3^{=} - \frac{2pPO_4^{\equiv}}{3} = pK'_{s.p.\ CaCO_3} - \frac{pK'_{s.p.\ Ca_3(PO_4)_2}}{3}$$

This equation shows that in any solution in equilibrium with both solid salts, $CaCO_3$ and $Ca_3(PO_4)_2$, there will be a definite ratio between the $CO_3^{=}$ ion concentration and the $PO_4^{\equiv}$ ion concentration; and one and only one ratio is compatible with equilibrium for both salts. Furthermore, fixing either the $[CO_3^{=}]$ or the $[PO_4^{\equiv}]$ fixes the $[Ca^{++}]$ which can be in equilibrium with the solid phase at that ionic strength.

One may fix the system in any one of a variety of ways. We have centered our attention on fixing the $p\alpha_{H^+}$ within physiological limits by adjusting the CO_2 tension. This has fixed the $[CO_3^{=}]$ which in turn fixed the $[Ca^{++}]$. Where phosphate was also

present, there must have been a further adjustment until the ratio of $\frac{[CO_3^{=}]}{[PO_4^{\equiv}]^{\frac{2}{3}}}$ satisfied the requirements of equilibrium.

EXPERIMENTAL RESULTS.

Effect of Varying Ionic Strength on Apparent or Stoichiometric Solubility Products of $CaCO_3$ and $Ca_3(PO_4)_2$.

Salt solutions were made up as outlined in the previous papers. Each solution contained calcium, phosphate, and bicarbonate with 1 per cent of volume added in weight as $CaCO_3$ and $Ca_3(PO_4)_2$. Eight different solutions were prepared, the ionic strength being increased in the second and third by $NaHCO_3$, after which the $NaHCO_3$ concentration was kept constant and the ionic strength increased with NaCl. The experiment was repeated (Series A) by preparing eight new solutions. Calcium was analyzed in 5 cc. samples and titrated with N/200 $KMnO_4$. The results given by electrometric $p\alpha_{H^+}$ determinations were all so unsatisfactory, apparently due to the electrode "poisoning" encountered by Holt, La Mer, and Chown, that the $p\alpha_{H^+}$ was calculated on the basis of total CO_2 and CO_2 tension analyses as outlined in the section on calculations.

The analytical determinations of calcium gave the value of $[Ca^{++}]$ immediately, since the difficultly soluble salts are assumed to be completely ionized. For each of the sixteen solutions, $p\alpha_{H^+}$ was calculated by equation (50), Paper I, by using the proper values for α_{CO_2} in accordance with equation (48). From the total $[CO_2]$, values for $[CO_3^{=}]$ and $[HCO_3^{-}]$ were obtained by equations (32) and (33), respectively. From the total $[PO_4]$ values for $[PO_4^{\equiv}]$ and $[HPO_4^{=}]$ were obtained by equations (16) and (15), Paper II, respectively.

Tables I and II contain the data obtained in these experiments. They have been plotted in Figs. 1 and 2 with $pK'_{s.p.}CaCO_3$ and $pK'_{s.p.}Ca_3(PO_4)_2$ as ordinates, and $\sqrt{\mu}$ as abscissæ. The equation of the line through these points was obtained in the following manner.

In order to calculate $pK'_{s.p.}$ of $CaCO_3$ and $Ca_3(PO_4)_2$ accurately, it was necessary to employ the stoichiometric values for the two dissociation constants of carbonic acid, and the stoichiometric

TABLE I.

Variation of $pK'_{s.p.}$ $CaCO_3$ at 38° in Salt Solutions of Varying Ionic Strength.

$pK_1'' = 6.33 - 0.5\sqrt{\mu} - 0.085\mu.$

$\alpha^0_{CO_2} = 0.557 - 0.103\mu.$

Experiment No.	pCO_2, mm.	$\alpha^0_{CO_2}$	$\frac{[H_2CO_3]\ M \times 10^3}{kg.\ H_2O}$	$\frac{\text{Total } [CO_2]\ M \times 10^3}{kg.\ H_2O}$	$\frac{[HCO_3^-] + [CO_3^=]\ M \times 10^3}{kg.\ H_2O}$	$\frac{[HCO_3^-] + [CO_3^=]}{[H_2CO_3]}$	Log ratio.	$p\alpha_{H^+}$ (calculated).	pK_1''	$\frac{[HCO_3^-]\ M \times 10^3}{kg.\ H_2O}$	$\frac{[CO_3^=]\ M \times 10^3}{kg.\ H_2O}$	$\frac{[Ca^{++}]\ M \times 10^3}{kg.\ H_2O}$	pCa^{++}	$pCO_3^=$	$pK'_{s.p.}$ $CaCO_3$	μ	$\sqrt{\mu}$
1	24.3	0.556	0.794	10.30	9.51	11.98	1.079	7.35	6.274	9.49	0.0168	0.452	3.345	4.774	8.12	0.0130	0.114
1A	22.3	0.556	0.728	10.24	9.51	13.01	1.114	7.39	6.274	9.49	0.0186	0.408	3.389	4.730	8.12	0.0129	0.114
2	65.0	0.555	2.120	20.86	18.74	8.84	0.947	7.20	6.253	18.73	0.0265	0.380	3.420	4.576	8.00	0.0226	0.150
2A	44.4	0.555	1.448	20.17	18.72	12.93	1.112	7.37	6.253	18.68	0.0391	0.268	3.572	4.407	7.98	0.0225	0.150
3	61.6	0.554	2.006	30.17	28.16	14.04	1.147	7.38	6.234	28.10	0.066	0.190	3.721	4.18	7.90	0.0323	0.180
4	57.9	0.552	1.877	30.32	28.44	15.16	1.181	7.39	6.208	28.36	0.078	0.229	3.640	4.11	7.75	0.0526	0.229
4A	64.2	0.551	2.078	32.85	30.77	14.81	1.171	7.38	6.208	30.69	0.083	0.210	3.678	4.08	7.76	0.0550	0.235
5	55.1	0.550	1.781	29.98	28.20	15.84	1.200	7.39	6.189	28.11	0.085	0.238	3.623	4.07	7.69	0.0726	0.269
5A	60.1	0.549	1.938	32.51	30.57	15.77	1.198	7.39	6.189	30.48	0.092	0.233	3.633	4.04	7.67	0.0751	0.274
6	53.6	0.546	1.720	29.67	27.95	16.25	1.211	7.37	6.161	27.86	0.090	0.273	3.564	4.05	7.61	0.1029	0.321
6A	57.1	0.546	1.832	32.64	30.81	16.82	1.226	7.39	6.161	30.70	0.104	0.226	3.646	3.98	7.63	0.1055	0.325
7	51.0	0.542	1.624	29.50	27.88	17.17	1.235	7.36	6.128	27.77	0.103	0.306	3.514	3.98	7.49	0.1435	0.379
7A	53.2	0.542	1.694	32.21	30.52	18.02	1.256	7.38	6.128	30.40	0.118	0.273	3.564	3.93	7.49	0.1460	0.382
8	46.5	0.538	1.469	29.66	28.19	19.19	1.283	7.38	6.100	28.07	0.125	0.333	3.478	3.90	7.38	0.1843	0.429
8A	49.1	0.538	1.551	29.36	27.81	17.93	1.254	7.35	6.100	27.69	0.115	0.342	3.466	3.94	7.41	0.1849	0.430

values for the three dissociation constants of phosphoric acid, which corresponded with the ionic strength for which one was to calculate $pK'_{s.p.}$ The data for the carbonic acid constants and

TABLE II.

Variation of $pK'_{s.p.}$ $Ca_3(PO_4)_2$ at 38° in Salt Solutions of Varying Ionic Strength.

$$pK_1' = 2.11 - 0.5\sqrt{\mu}$$
$$pK_2' = 7.15 - 1.25\sqrt{\mu}$$
$$pK_3' = 12.66 - 2.25\sqrt{\mu}$$

Experiment No.	pαH+ (calculated).	Total $[PO_4]$ $\frac{M \times 10^4}{kg.\ H_2O}$	$[PO_4{\equiv}]$ $\frac{M \times 10^5}{kg.\ H_2O}$	$[Ca^{++}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	pCa++	$pPO_4{\equiv}$	$pK'_{s.p.}$ $Ca_3(PO_4)_2$	μ	$\sqrt{\mu}$
1	7.35	0.37	0.214	0.452	3.345	9.669	29.37	0.0130	0.114
1A	7.39	0.40	0.262	0.408	3.389	9.582	29.33	0.0129	0.114
2	7.20	1.42	0.668	0.380	3.420	9.175	28.61	0.0226	0.150
2A	7.37	1.45	1.14	0.268	3.572	8.943	28.60	0.0225	0.150
3	7.38	2.29	2.23	0.190	3.721	8.652	28.47	0.0323	0.180
4	7.39	2.51	3.35	0.229	3.640	8.475	27.87	0.0526	0.229
4A	7.38	3.02	4.12	0.210	3.678	8.385	27.80	0.0550	0.235
5	7.39	2.43	4.10	0.238	3.623	8.387	27.64	0.0726	0.269
5A	7.39	2.80	4.97	0.233	3.633	8.304	27.51	0.0751	0.274
6	7.37	2.55	5.53	0.273	3.564	8.257	27.21	0.1029	0.321
6A	7.39	3.06	7.19	0.226	3.646	8.143	27.22	0.1055	0.325
7	7.36	2.89	8.48	0.306	3.514	8.072	26.69	0.1435	0.379
7A	7.38	3.02	9.60	0.273	3.564	8.018	26.73	0.1460	0.382
8	7.38	2.59	10.77	0.333	3.478	7.968	26.37	0.1843	0.429
8A	7.35	2.70	10.43	0.342	3.466	7.982	26.36	0.1849	0.430

those for phosphoric acid were obtained from various sources described in the preceding papers in sections devoted to the subject.

Upon plotting the experimental points for $pK'_{s.p.}$ against the square root of the ionic strength it was seen that the curves for carbonate and phosphate deviated slightly from a straight line. The lowest ionic strength in these experiments being a little over

0.01 ($\sqrt{\mu}$ = 0.114), a graphical extrapolation to zero ionic strength seemed a questionable procedure. In order, however, to check it up with theory, the following treatment was employed to locate the activity solubility product, $pK'_{s.p.}$, and at the same time to determine the best theoretical curve, running from the solubility product at zero ionic strength, through the stoichiometric solubility products at increasing salt concentrations.

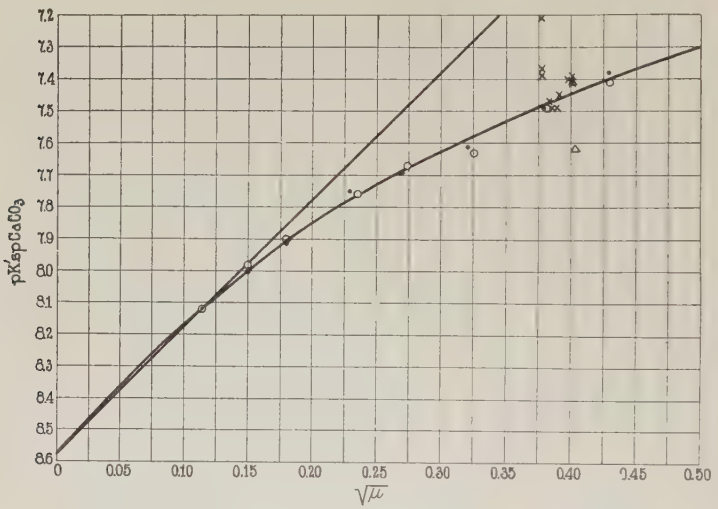


FIG. 1. The solubility of $CaCO_3$ in salt solutions of varying ionic strength saturated with $CaCO_3$ and $Ca_3(PO_4)_2$ at 38°. Values of $pK'_{s.p.}CaCO_3$ are plotted as ordinates and $\sqrt{\mu}$ as abscissæ. The curved line has the equation $pK'_{s.p.}CaCO_3 = 8.58 - \frac{4.94\sqrt{\mu}}{1 + 1.85\sqrt{\mu}}$.

According to Hückel, the activity coefficient of strong electrolytes varies with the ionic strength in accordance with a formula which has the general form $-\log \gamma_{salt} = \frac{B\sqrt{\mu}}{1 + A\sqrt{\mu}} + C\mu$ (see equations (20) and (21), Paper I) where B, A, and C are constants depending, among other factors, upon the electronic environment, secondary electrical effects such as "salting out" or hydration, the average size of the ions, and the change in dielectric constant of the solvent with salt. In the case of such

slightly soluble salts as $CaCO_3$ and $Ca_3(PO_4)_2$, one may assume complete dissociation and apply directly the theory of strong electrolytes as presented in the section on theory. In the case of weak electrolytes, which obey the classical law of mass action, the ions must of necessity be subject to the same influences as those of strong electrolytes.

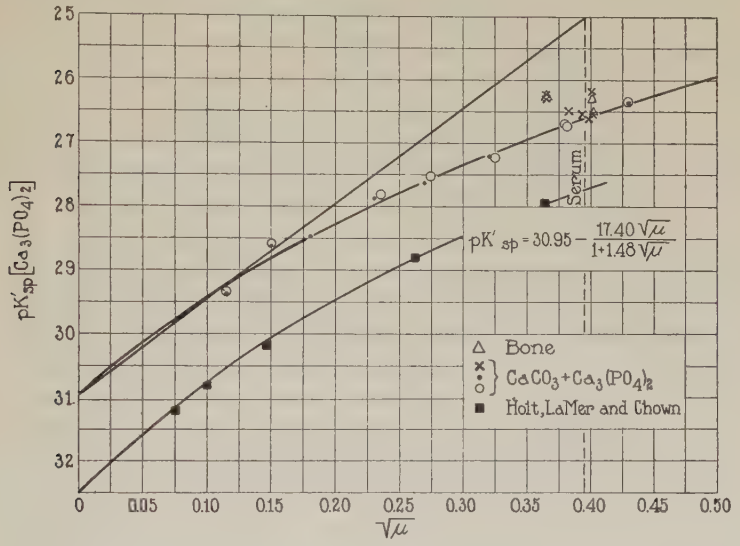


FIG. 2. The solubility of $Ca_3(PO_4)_2$ in salt solutions of varying ionic strength saturated with $CaCO_3$ and $Ca_3(PO_4)_2$ at 38°. Values of $pK'_{s.p.}$ $Ca_3(PO_4)_2$ are plotted as ordinates and $\sqrt{\mu}$ as abscissæ. The curved line has the equation $pK'_{s.p.}Ca_3(PO_4)_2 = 30.95 - \frac{17.40\sqrt{\mu}}{1 + 1.48\sqrt{\mu}}$.

Calculation of $pK'_{s.p.}CaCO_3$.—According to the equations of Paper I,

$$\alpha_{Ca^{++}} \times \alpha_{CO_3^{=}} = K_{s.p.}\ CaCO_3 \tag{6}$$

$$\gamma_{Ca^{++}}[Ca^{++}] \times \gamma_{CO_3^{=}}[CO_3^{=}] = K_{s.p.}\ CaCO_3 \tag{7}$$

$$[Ca^{++}][CO_3^{=}] = \frac{K_{s.p.}\ CaCO_3}{\gamma_{Ca^{++}} \times \gamma_{CO_3^{=}}} = K'_{s.p.}\ CaCO_3 \tag{8}$$

or $pK_{s.p.}\ CaCO_3 = pK'_{s.p.}\ CaCO_3 + p\ \gamma_{Ca^{++}} + p\ \gamma_{CO_3^{=}}$

But since by definition, the activity coefficient of a salt is the geometrical mean of the activity coefficients of its ions,

$$(9) \qquad \frac{p\gamma_{Ca^{++}} + p\gamma_{CO_3^{=}}}{2} = p\gamma_{CaCO_3}$$

$$(10) \qquad \text{or } pK_{s.p.}\ CaCO_3 - pK'_{s.p.}\ CaCO_3 = 2\ p\gamma_{CaCO_3}$$

$$(11) \qquad \Delta pK'_{s.p.}\ CaCO_3 = 2\ p\gamma_{CaCO_3}$$

According to Brönsted and La Mer (1924)

$$\frac{\Delta pK'_{s.p.}}{\nu} = -\log_{10}\gamma_{salt} = \alpha' z_1 z_2 \sqrt{\mu}$$

where ν = number of ions of the salt, α' is a universal constant, and z_1 and z_2 are the valences of the ions. Hence

$$(12) \qquad \Delta\ pK'_{s.p.}\ CaCO_3 = 2p\gamma_{CaCO_3} = 4\sqrt{\mu}$$

Their expression holds for highly dilute solutions only, but by the addition of another term $0.87\beta c$, it may be corrected so that it is valid to a concentration of $\mu = 0.2$. For higher concentrations, the formula of Hückel (1925) is better suited, and we have accordingly adopted it, omitting the correction factor $C\mu$.

Drawing graphically an approximately average curve through the sixteen points for $pK'_{s.p.}\ CaCO_3$ and extrapolating to zero ionic strength, $pK'_{s.p.}\ CaCO_3$ was found to be approximately 8.6. Since equation (12) holds for dilute solutions, it was applied in its simplest form to the points of lowest ionic strength, namely 1, 1A, 2, 2A. These gave the average value 8.58 for $pK_{s.p.}\ CaCO_3$, which agreed with that found in experiments already described with only $CaCO_3$ as the saturating body. This figure was then used as the basis of further calculations to obtain the constants of equation (18). Of course, the two methods of extrapolation, although they agree quite well, afford no assurance that the solubility product at infinite dilution is any more accurate than to about 0.1. However, the value found, arbitrary though it may be, serves its purpose. It furnishes the basis for calculating the expression representative of the empirical points found, as placed on a theoretical curve.

According to equations (21), Paper I, and (11),

$$(13) \qquad p\gamma_{CaCO_3} = \frac{B\sqrt{\mu}}{1 + A\sqrt{\mu}} = \frac{\Delta\ pK'_{s.p.}\ CaCO_3}{2}$$

By inversion and separation into different terms,

$$(14) \qquad \frac{2}{\Delta\ pK'_{s.p.}\ CaCO_3} = \frac{1 + A\sqrt{\mu}}{B\sqrt{\mu}} = \frac{1}{B\sqrt{\mu}} + \frac{A}{B}$$

Thus, when plotting $\frac{2}{\Delta\ pK'_{s.p.}\ CaCO_3}$ against $\frac{1}{\sqrt{\mu}}$, a line can be drawn which determines the empirical constants B and A, the slope being $\frac{1}{B}$, the intercept $\frac{A}{B}$. Thus

$$\frac{2}{\Delta\ pK'_{s.p.}\ CaCO_3} = \frac{1 + 1.85\sqrt{\mu}}{2.47\sqrt{\mu}} \tag{15}$$

or

$$p\gamma_{CaCO_3} = \frac{2.47\sqrt{\mu}}{1 + 1.85\sqrt{\mu}} = \frac{\Delta\ pK'_{s.p.}\ CaCO_3}{2} \tag{16}$$

or

$$2p\gamma_{CaCO_3} = \frac{4.94\sqrt{\mu}}{1 + 1.85\sqrt{\mu}} = \Delta pK'_{s.p.}\ CaCO_3 = p\gamma_{Ca^{++}} + p\gamma_{CO_3^{=}} \tag{17}$$

Hence, by equations (10) and (17), the equation for the theoretical line through the experimental points will be

$$pK'_{s.p.}\ CaCO_3 = 8.58 - \frac{4.94\sqrt{\mu}}{1 + 1.85\sqrt{\mu}} \tag{18}$$

Table III shows how those points fit the calculated curve. Of course, they must fit fairly well, since the constants B and A were derived by using them in calculations. However, the possibility of working in such a manner with the results recorded may be regarded as an index of the accuracy of the experimental work.

We cannot compare our estimations directly with those of Johnston because of the fact that our measurements were carried out at 38°, whereas his were made at 16°. From the work of Leather and Sen (1909), Johnston (1915) calculated that the solubility product of calcium carbonate at 40° was 0.5×10^{-8}, or $pK'_{s.p.}CaCO_3 = 8.30$. The ionic strength of the solution was approximately 0.005 which corresponds to the value 8.29 for $pK'_{s.p.}CaCO_3$ on Fig. 1. This is cited to show that the order of magnitude of our values is not inconsistent with what has been found in past studies on $CaCO_3$ solubility.

Attention may be called here to the difference in the two curves

for $pK'_{s.p.}CaCO_3$ obtained when salt solutions are saturated with $CaCO_3$ in the presence and absence of $Ca_3(PO_4)_2$. By equation (63), Paper I, when $\mu = 0.16$, $pK'_{s.p.}CaCO_3 = 7.38$, while equation (18) gives $pK'_{s.p.}CaCO_3 = 7.44$. As can be seen for equations (18) and (63), Paper I, the effect is marked only at the higher concentrations and is apparently real, for the averages of

TABLE III.

Comparison of Observed and Calculated $pK'_{s.p.}CaCO_3$ at 38°, at Various Ionic Strengths.

Experiment No.	$\sqrt{\mu}$	$pK'_{s.p.}$ calculated.	$pK'_{s.p.}$ observed.	$pK'_{s.p.}$ deviation.
1	0.114	8.12	8.12	0.00
1A	0.114	8.12	8.12	0.00
2	0.150	8.00	8.00	0.00
2A	0.150	8.00	7.98	−0.02
3	0.180	7.91	7.90	−0.01
4	0.229	7.78	7.75	−0.03
4A	0.235	7.77	7.76	−0.01
5	0.269	7.69	7.69	0.00
5A	0.274	7.68	7.67	−0.01
6	0.321	7.58	7.61	+0.03
6A	0.325	7.58	7.63	+0.05
7	0.379	7.48	7.49	+0.01
7A	0.382	7.48	7.49	+0.01
8	0.429	7.40	7.38	−0.02
8A	0.430	7.40	7.41	+0.01

the several different determinations at this region under the two conditions also give values which are distinctly different. Apparently, the presence of $PO_4^{\equiv}$ ion, of unsymmetrical valence type with respect to Ca^{++} ion, exerts some secondary salt effect. As a consequence of the theory of strong electrolytes from the standpoint of electronic forces between ions and their surrounding environment, this discrepancy might be expected. It is unknown,

however, whether the slight amount of phosphate in these solutions could account for the differences in the two curves.

Calculation of $pK'_{s.p.}\ Ca_3(PO_4)_2$.—This development is analogous to the one for calcium carbonate:

(2) $$(\alpha_{Ca^{++}})^3 \times (\alpha_{PO_4^{\equiv}})^2 = K_{s.p.}\ Ca_3(PO_4)_2$$

(19) $$(\gamma_{Ca^{++}})^3\,[Ca^{++}]^3 \times (\gamma_{PO_4^{\equiv}})^2\,[PO_4^{\equiv}]^2 = K_{s.p.}\ Ca_3(PO_4)_2$$

(20) $$[Ca^{++}]^3 \times [PO_4^{\equiv}]^2 = \frac{K_{s.p.}\ Ca_3(PO_4)_2}{(\gamma_{Ca^{++}})^3\,(\gamma_{PO_4^{\equiv}})^2} = K'_{s.p.}\ Ca_3(PO_4)_2$$

or, transforming to logarithmic form,

(21) $$pK_{s.p.}\ Ca_3(PO_4)_2 = pK'_{s.p.}\ Ca_3(PO_4)_2 + 3\ p\gamma_{Ca^{++}} + 2\ p\gamma_{PO_4^{\equiv}}$$

(22) $$\frac{3\ p\gamma_{Ca^{++}} + 2\ p\gamma_{PO_4^{\equiv}}}{5} = p\gamma_{Ca_3(PO_4)_2}$$

Hence,

(23) $$pK_{s.p.}\ Ca_3(PO_4)_2 - pK'_{s.p.}\ Ca_3(PO_4)_2 = 5\ p\gamma_{Ca_3(PO_4)_2}$$

(24) $$\Delta\ pK'_{s.p.}\ Ca_3(PO_4)_2 = 5\ p\gamma_{Ca_3(PO_4)_2}$$

By equation (19), Paper I,

(25) $$\Delta\ pK'_{s.p.}\ Ca_3(PO_4)_2 = 5\ p\gamma_{Ca_3(PO_4)_2} = 15\sqrt{\mu}$$

for dilute solutions. Extrapolating back graphically, and with the aid of equation (25) from points of lowest ionic strength 1, 1A, 2, and 2A, the activity solubility product at zero ionic strength was found to be 30.95. Such an extrapolation involves an error in $pK_{s.p.}\ Ca_3(PO_4)_2$ of probably 0.3.

Although Hückel's equation has not been applied to salts of unsymmetrical higher valence type, in the absence of any other method that would yield a better expression for the variation of $pK'_{s.p.}\ Ca_3(PO_4)_2$ with ionic strength, it was decided to employ such an equation to fit the data, as had already been done for calcium carbonate. With this reservation, the calculations were performed as before.

(26) $$p\gamma_{Ca_3(PO_4)_2} = \frac{B\sqrt{\mu}}{1 + A\sqrt{\mu}} = \frac{\Delta pK'_{s.p.}\ Ca_3(PO_4)_2}{5}$$

(27) $$\frac{5}{\Delta\ pK'_{s.p.}\ Ca_3(PO_4)_2} = \frac{1 + A\sqrt{\mu}}{B\sqrt{\mu}} = \frac{1}{B\sqrt{\mu}} + \frac{A}{B}$$

Plotting $\frac{5}{\Delta\ pK'_{s.p.\ Ca_3(PO_4)_2}}$ against $\frac{1}{\sqrt{\mu}}$ as before,

B = 3.48 and A = 1.48. Accordingly, equation (26) becomes

$$(28) \qquad p\gamma_{Ca_3(PO_4)_2} = \frac{3.48\sqrt{\mu}}{1 + 1.48\sqrt{\mu}} = \frac{\Delta\ pK'_{s.p.\ Ca_3(PO_4)_2}}{5}$$

TABLE IV.

Comparison of Observed and Calculated $pK'_{s.p.}$ $Ca_3(PO_4)_2$ at 38°, at Various Ionic Strengths.

Experiment No.	$\sqrt{\mu}$	$pK'_{s.p.}$ calculated.	$pK'_{s.p.}$ observed.	$pK'_{s.p.}$ deviation.
1	0.114	29.25	29.37	+0.12
1A	0.114	29.25	29.33	+0.08
2	0.150	28.82	28.61	−0.21
2A	0.150	28.82	28.60	−0.22
3	0.180	28.48	28.47	−0.01
4	0.229	27.97	27.87	−0.10
4A	0.235	27.92	27.80	−0.12
5	0.269	27.60	27.64	+0.04
5A	0.274	27.56	27.51	−0.05
6	0.321	27.16	27.21	+0.05
6A	0.325	27.13	27.22	+0.09
7	0.379	26.73	26.69	−0.04
7A	0.382	26.70	26.73	+0.03
8	0.429	26.39	26.37	−0.02
8A	0.430	26.38	26.36	−0.02

or

$$(29) \qquad 5\ p\gamma_{Ca_3(PO_4)_2} = \frac{17.40\sqrt{\mu}}{1 + 1.48\sqrt{\mu}} = \Delta\ pK'_{s.p.\ Ca_3(PO_4)_2} = 3\ p\gamma_{Ca^{++}} + 2\ p\gamma_{PO_4^{\equiv}}$$

Hence, by equations (29) and (21) the equation for the theoretical line through the experimental points will be

$$(30) \qquad pK'_{s.p.\ Ca_3(PO_4)_2} = 30.95 - \frac{17.40\sqrt{\mu}}{1 + 1.48\sqrt{\mu}}$$

Table IV shows the difference between actual experimental points and the calculated curve.

At this point, it may be interesting to compare the foregoing results with the curve for $pK'_{s.p.}Ca_3(PO_4)_2$ obtained by Holt, La Mer, and Chown ((1925) p. 544), in which the ionic strength was varied by means of KCl and NaCl. They apparently did not distinguish between K, the dissociation constant at infinite dilution, and K', the stoichiometric or apparent dissociation constant of an acid. Consequently, it may be expected that the use of the three dissociation constants of phosphoric acid at the same value throughout would give a curve which would be differ-

TABLE V.

Variation in $pK'_{s.p.}$ $Ca_3(PO_4)_2$ at 38° with Varying Ionic Strength.

Recalculated from Holt, La Mer, and Chown (1925, *a*), Table V, p. 538. $Ca(OH)_2+H_3PO_4$, shaking 8 days.

Experiment No.	p_{H^+}	$[Ca^{++}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[H_2PO_4^-]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[HPO_4^=]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[PO_4^{\equiv}]$ $\frac{M \times 10^{12}}{kg.\ H_2O}$	pCa^{++}	$pPO_4^{\equiv}$	$pK'_{s.p.}$ $Ca_3(PO_4)_2$	μ	$\sqrt{\mu}$
1	5.21	1.96	3.71	3.66	0.053	2.84	2.707	11.547	31.22	0.0058	0.076
2	5.27	1.98	3.63	3.57	0.064	4.38	2.703	11.358	30.82	0.0098	0.099
3	5.33	2.00	3.58	3.49	0.084	8.99	2.699	11.046	30.19	0.0216	0.147
4	5.46	2.07	3.54	3.39	0.155	41.62	2.684	10.381	28.81	0.0690	0.263
5	5.51	2.12	3.54	3.33	0.214	98.2	2.673	10.007	28.03	0.1324	0.364
6	5.52	2.22	3.59	3.37	0.223	104	2.653	9.983	27.93	0.1326	0.364
7	5.53	3.11	4.04	3.65	0.391	428	2.507	9.368	26.26	0.2618	0.512

ent from one that would be obtained by the use of constants, which have been corrected for the known increase which takes place with increasing concentration. Accordingly, their values have been recalculated using the constants as given in the preceding paper, and placing their concentrations on a molality instead of a molarity basis. This was done by using a water content which varied with the concentration according to the approximate formula obtained at room temperature at this laboratory; namely,

$$(996 - 30\,\mu) = \text{gm. of } H_2O \text{ per liter.} \tag{31}$$

The results are shown in Table V and Fig. 2.

It is at once evident that the two curves differ considerably in

the value for $pK'_{s.p.}Ca_3(PO_4)_2$ at zero ionic strength, their curve giving on extrapolation, $pK_{s.p.}\ Ca_3(PO_4)_2 = 32.50$ as compared with our value of 30.95.

Inasmuch as the two sets of data were obtained from two different systems, it is impossible to tell whether technique or some other factor is the source of the discrepancy. The experiments recorded in these papers were done in the presence of solid $CaCO_3$ and $Ca_3(PO_4)_2$; theirs were obtained by adding $Ca(OH)_2$ to H_3PO_4 and shaking for 8 days to permit complete precipitation. In these particular systems, when the solid is present initially one might expect to attain equilibrium sooner than when waiting for the solid to precipitate. Their reaction ($p\alpha_{H^+} = 5.21$ to 5.53) resulted in a much smaller concentration of $PO_4^{\equiv}$ ion and a correspondingly greater concentration of $HPO_4^{=}$, $H_2PO_4^{-}$, and Ca^{++} ions. In view of their demonstration of the specific effect of excess $H_2PO_4^{-}$ ion on $pK'_{s.p.}Ca_3(PO_4)_2$, it is difficult to understand why that ion did not exert a similar effect in these solutions. Even in the presence of NaCl and KCl, the tendency to increase the stoichiometric solubility product with increasing concentration, should have been much more than it was. Their $p\alpha_{H^+}$ values, on the basis of N/10 HCl = 1.04 would make $pK'_{s.p.}$ $Ca_3(PO_4)_2$ even greater by 0.08 or 0.09. Inasmuch as it is not known whether there was any correction made for diffusion potential, their data have been recalculated at their original $p\alpha_{H^+}$ values.

Activity Coefficients of $CaCO_3$, $Ca_3(PO_4)_2$, and Ca^{++} Ion in Salt Solutions of Varying Ionic Strength.—The experiments on the variation in stoichiometric solubility product of $CaCO_3$ and $Ca_3(PO_4)_2$ with varying salt concentration, enable one to calculate the activity coefficient of the salts. By utilizing the activity coefficients of the $CO_3^{=}$ ion and the $PO_4^{\equiv}$ ion given in the section on constants and equations, one may calculate the activity coefficient of Ca^{++} ion and its variation with ionic strength.

By definition, the activity coefficient of a salt is the geometrical mean of the activity coefficients of its ions. Thus

$$(\gamma_{Ca^{++}})^3 \times (\gamma_{PO_4^{\equiv}})^2 = (\gamma_{Ca_3(PO_4)_2})^5 \text{ or}$$

(22) $$3\ p\gamma_{Ca^{++}} + 2\ p\gamma_{PO_4^{\equiv}} = 5\ p\gamma_{Ca_3(PO_4)_2}$$

Therefore, it will be necessary to evaluate the activity coefficient variation with ionic strength, for the anion of the particular calcium salt being studied. According to Hastings and Sendroy (1925)

(32) $$p\gamma_{CO_3^{=}} = 1.6\sqrt{\mu}$$

(9) $$2\,p\gamma_{CaCO_3} = p\gamma_{Ca^{++}} + p\gamma_{CO_3^{=}}$$

By substituting the value of $2\,p\gamma_{CaCO_3}$ from equation (9)

(33) $$p\gamma_{Ca^{++}} = 2\,p\gamma_{CaCO_3} - p\gamma_{CO_3^{=}} = \frac{4.94\sqrt{\mu}}{1 + 1.85\sqrt{\mu}} - 1.6\sqrt{\mu}$$

or

(34) $$p\gamma_{Ca^{++}} = \left(\frac{4.94}{1 + 1.85\sqrt{\mu}} - 1.6\right)\sqrt{\mu}$$

In the same way, using $p\gamma_{PO_4^{\equiv}}$ as derived in Paper II, we have

(28) $$p\gamma_{PO_4^{\equiv}} = 4.0\sqrt{\mu}$$

(22) $$5\,p\gamma_{Ca_3(PO_4)_2} = 3\,p\gamma_{Ca^{++}} + 2\,p\gamma_{PO_4^{\equiv}}$$

(35) $$p\gamma_{Ca^{++}} = \tfrac{5}{3}\,p\gamma_{Ca_3(PO_4)_2} - \tfrac{2}{3}\,p\gamma_{PO_4^{\equiv}}$$

$$= \tfrac{5}{3}\left(\frac{3.48\sqrt{\mu}}{1 + 1.48\sqrt{\mu}}\right) - \tfrac{2}{3}(4.0\sqrt{\mu})$$

$$= \frac{5.80\sqrt{\mu}}{1 + 1.48\sqrt{\mu}} - 2.67\sqrt{\mu}$$

or

(36) $$p\gamma_{Ca^{++}} = \left(\frac{5.80}{1 + 1.48\sqrt{\mu}} - 2.67\right)\sqrt{\mu}$$

Table VI and Fig. 3 represent the values for the activity coefficients of $CaCO_3$ and $Ca_3(PO_4)_2$ varying with ionic strength, calculated according to the equations of the preceding paragraphs. Column 5 gives Ca^{++} ion activity coefficient values from the $CaCO_3$ solubility data, while those of Column 9 are derived from

the corresponding data on $Ca_3(PO_4)_2$. Examination of the carbonate data reveals the fact that even at the lowest concentrations, the activity coefficients of $CO_3^=$ ion and Ca^{++} ion are different. This is in accord with what little evidence has so far been obtained on individual ion activities. The activity coeffi-

TABLE VI.

Variation with Ionic Strength of the Activity Coefficients of $CaCO_3$, $Ca_3(PO_4)_2$, and Ca^{++} Ion at 38°.

Experiment No.	$p\gamma_{CaCO_3}$	γ_{CaCO_3}	$p\gamma_{Ca^{++}}$	$\gamma_{Ca^{++}}$	$p\gamma_{Ca_3(PO_4)_2}$	$\gamma_{Ca_3(PO_4)_2}$	$p\gamma_{Ca^{++}}$	$\gamma_{Ca^{++}}$	μ	$\sqrt{\mu}$
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
1	0.230	0.588	0.278	0.527	0.340	0.457	0.263	0.546	0.0130	0.114
1A	0.230	0.588	0.278	0.527	0.340	0.457	0.263	0.546	0.0129	0.114
2	0.290	0.512	0.340	0.457	0.426	0.375	0.307	0.493	0.0226	0.150
2A	0.290	0.512	0.340	0.457	0.426	0.375	0.307	0.493	0.0225	0.150
3	0.335	0.462	0.382	0.415	0.494	0.320	0.343	0.454	0.0323	0.180
4	0.400	0.398	0.434	0.368	0.596	0.253	0.382	0.415	0.0526	0.229
4A	0.405	0.402	0.434	0.368	0.606	0.248	0.383	0.414	0.0550	0.235
5	0.445	0.359	0.460	0.347	0.670	0.214	0.399	0.399	0.0726	0.269
5A	0.450	0.354	0.462	0.345	0.678	0.210	0.398	0.400	0.0751	0.274
6	0.500	0.316	0.486	0.326	0.758	0.175	0.407	0.392	0.1029	0.321
6A	0.500	0.316	0.480	0.331	0.764	0.172	0.406	0.392	0.1055	0.325
7	0.550	0.282	0.494	0.320	0.844	0.143	0.395	0.403	0.1435	0.379
7A	0.550	0.282	0.489	0.324	0.850	0.141	0.397	0.401	0.1460	0.382
8	0.590	0.257	0.494	0.320	0.912	0.122	0.374	0.422	0.1843	0.429
8A	0.590	0.257	0.492	0.322	0.914	0.122	0.375	0.421	0.1849	0.430

cients of the cation and anion in solutions of a difficultly soluble salt, do not necessarily have to be equal, even when cation and anion are of similar valence type. With increasing ionic strength, one would expect such differences to increase, as they do in these experiments.

Lewis and Randall (p. 362) have collected into one table the activity coefficients of typical electrolytes as established by freezing point data. We reproduce here their values for the molality of 0.01 followed by our own values for $CaCO_3$ and $Ca_3(PO_4)_2$ calculated at the same concentration.

HCl (25°)	LiCl (25°) NaCl (25°) KCl (25°)	KOH (25°)	KNO_3	$AgNO_3$	KIO_3 $NaIO_3$	$BaCl_2$
0.924	0.922	0.92	0.916	0.902	0.882	0.716
$CdCl_2$ (25°)	K_2SO_4	H_2SO_4 (25°)	$La(NO_3)_3$ $K_3Fe(CN)_6$ (p. 344)	$MgSO_4$ $CdSO_4$ $CuSO_4$	$CaCO_3$ (38°)	$Ca_3(PO_4)_2$ (38°)
0.532	0.687	0.617	0.571	0.404	0.436	0.139

Here, apparently, the activity coefficient decreases as the complexity of the salt grows with increasing number of ions in the molecule, increasing valence of the ions, and dissimilarity in pairs of ions constituting the salt. The activity coefficients of $CaCO_3$ and $Ca_3(PO_4)_2$ at $c = 0.01$, were calculated according to equations (34) and (36) respectively.

The values for $CaCO_3$ and $Ca_3(PO_4)_2$ $pK_{s.p.}$ are those which would be given by our equations on the basis of μ in NaCl solutions. If only Ca^{++} and $CO_3^{=}$ ions or Ca^{++} and $PO_4^{\equiv}$ ions contributed to the entire ionic strength at these molal concentrations, *i.e.* if we could possibly have only a pure solution of $CaCO_3$ and $Ca_3(PO_4)_2$, perhaps these values would be different. As it is, not having such data, it has been necessary to resort to the calculation indicated above. The rule given by Lewis and Randall that the activity coefficient of a given electrolyte is the same in all solutions of the same ionic strength, is a limiting law and does not hold rigidly with increasing concentration and increasing complexity of the salt. As has been noted before, even at the same ionic strength, at a concentration such as these given here, the nature of the ions in solution has marked specific effects on the thermodynamic properties of such a solution. Bearing in mind that the activity coefficient of such a salt as $Ca_3(PO_4)_2$ may be quite different in a solution where only Ca^{++} and $PO_4^{\equiv}$ ions are present from one in which NaCl is predominant, we will note the relative activity coefficients of a few salts at $\mu = 0.04$. The

sulfate value is obtained from the same source as those above, the $CaCO_3$ and $Ca_3(PO_4)_2$ being calculated according to equations (34) and (36) respectively.

$MgSO_4$ $CdSO_4$ $CuSO_4$ $CaSO_4$ (p. 376)	0.404; $CaCO_3$ (38°), 0.436; $Ca_3(PO_4)_2$ (38°), 0.289

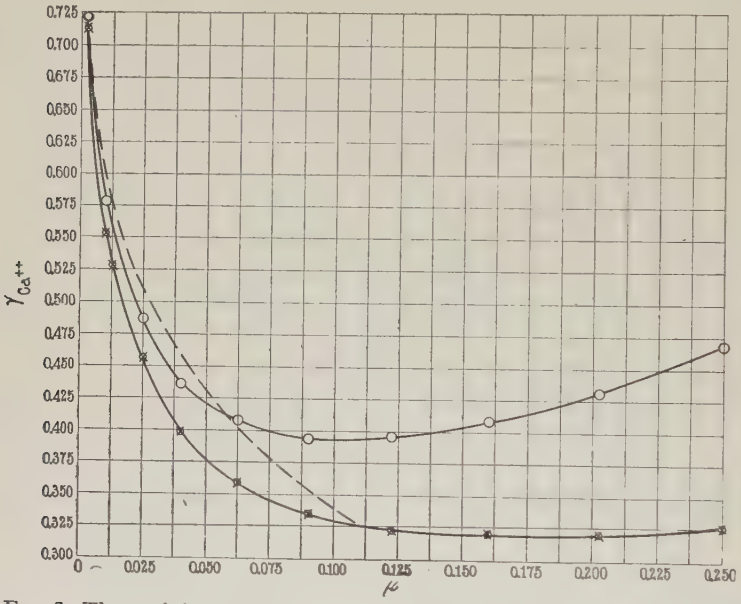


FIG. 3. The activity coefficient, γ, of calcium plotted against the ionic strength μ, calculated from $CaCO_3$ solubility data and from $Ca_3(PO_4)_2$ solubility data. The former are designated by ○, the latter by ⊗. The broken line is plotted from data given in Lewis and Randall's Thermodynamics, p. 382, for divalent ions.

The low value for $Ca_3(PO_4)_2$ may be due to the errors involved either in calculating for μ as we have noted above, in using doubtful dissociation constant values for phosphoric acid, or to a number of other unknown factors such as degree of hydration of the ions.

Considering the difficulties involved, it is not surprising to find that the activity coefficients of Ca^{++} ion, calculated from the solubility data of the two salts, are different. However, inasmuch

as both values have been obtained on exactly the same solutions, and the Ca^{++} ion activity at each concentration can have but one value, we must conclude that the discrepancy is a result of one of the factors mentioned above. Probably most of the difference represents the result of the combined error that may be involved in using the particular dissociation constants for carbonic acid and phosphoric acid which have been adopted for the calculations.

Fig. 3 indicates the variation of the activity coefficient, $\gamma_{Ca^{++}}$, with ionic strength. We have also plotted the few points of Lewis and Randall (p. 382) of average values for several bivalent cations, Ca^{++} included, calculated from the activity coefficient of the corresponding halides and that of Cl^- ion. These check our experimental results up to $\mu = 0.1$. Our results, however, show that the decrease in value of γ ceases at the low ionic strength of 0.1 in one case and of 0.2 in that of the other. This is quite unlike the behavior of the activity coefficient of Ca^{++} as determined from $CaSO_4$ data. Results such as these may be due to difference in electronic environment or inaccuracies in the use of the acid activity coefficients with varying μ.

To quote from Harned (1924), "It would appear that the activity coefficient of an ion at constant temperature is a function of its concentration, its valence, its dimensions, its complexity and its interaction with the other ions in the solution containing it. At higher concentrations, the effect of hydration becomes prominent as a factor which probably causes the thermodynamic behaviors of the ions to differ." It would seem that the difficulties attendant to the study of the activity of individual ions are enormous, and that many of the ideas on the subject are to be regarded as no more than tentative, awaiting further extension of our knowledge before further refinements in accuracy are possible. It is with this view-point that the material related to calcium ion and salt activities has been presented.

Solubility of $CaCO_3$ and $Ca_3(PO_4)_2$ in Salt Solutions under Various Conditions.—In order to obtain light on the question of the precipitation and solution of calcium salts in biological fluids, it was considered desirable to study the effect of the various conditions under which equilibrium with the two saturating salts $CaCO_3$ and $Ca_3(PO_4)_2$ could be attained. The effect of varying ionic strength has been described. The time limit of equilibrium

between solid and liquid phase, the possible variation in results with differences in the initial composition of the salt solutions, and incidentally the effect, if any, of differences in $p\alpha_{H^+}$ were investigated.

In order to simulate as nearly as possible the composition of serum without its protein, balanced salt solutions already described were used. It was found that, contrary to expectation, such an artificial edema fluid gave no evidence of specific effect of the other ions present, but behaved as if it were entirely a NaCl solution of the same ionic strength.

One of the first considerations was the question of the effect of such a solution containing the various ions on the activity coefficient of the bicarbonate ion. This had been determined previously only in the presence of NaCl and $NaHCO_3$. In view of the marked specific effect of higher valence type ions on the electronic properties of solutions, pK_1'' of carbonic acid was determined in four of the saturation systems, Nos. 10, 11, 14, and 15, and in another group in which artificial edema fluid was used without saturation with solid or addition of Ca^{++} ion. Calculations were made according to equation (50). The results are given in Table VII. It was found that the average value of the saturation system determinations gave $pK_1'' = 6.135$ and the other three gave 6.154. Hence it was felt that the use of $pK_1'' = 6.13$ on the basis of the determinations in NaCl solutions previously made by Hastings and Sendroy, was justified in the case of these solutions. Any error in using the wrong value of pK_1'' would be of no greater magnitude than the error involved in calculation with a similar discrepancy in $p\alpha_{H^+}$. Further, the $p\alpha_{H^+}$ of "30," "40," and "50" was determined both electrometrically and colorimetrically as indicated in the table, with practically no difference in results. This served as a check on the maintenance of the value of the apparent dissociation constant of phenol red which had previously been determined for the bicolor standards against M/15 phosphate solution. In view of these results, it was considered correct to apply all data on sodium chloride solutions of the same ionic strength to the artificial edema fluid used in these experiments.

Table VIII gives a general summary of the results of this group of experiments. The experiments have been separated into four

groups, according to the initial composition, and will be treated separately in that manner in the analysis which follows.

Group I. Experiments 9 and 14.—In this group calcium and phosphate were initially absent from the solutions. In order to satisfy the stoichiometric solubility product of both $CaCO_3$ and $Ca_3(PO_4)_2$, the two salts dissolved, furnishing Ca^{++}, $CO_3^{=}$, and $PO_4^{\equiv}$ ions. Any excess of Ca^{++} over the carbonate solubility product would cause reprecipitation as the carbonate. Inasmuch as in this case the amount of $Ca_3(PO_4)_2$ dissolved furnished just

TABLE VII.

Determination of pK_1'' H_2CO_3 in Artificial Edema Fluid.

Experiment No.	pCO_2, mm.	$\alpha^{0}_{CO_2}$	$[H_2CO_3]$ M × 10³ / kg. H_2O	Total $[CO_2]$ M × 10³ / kg. H_2O	$[HCO_3^-]$ M × 10³ / kg. H_2O	pcrH⁺	$\frac{[HCO_3^-]}{[H_2CO_3]} = R$	log R	pK_1''	μ	$\sqrt{\mu}$
"30"	30.9	0.541	0.982	30.97	29.83	7.633e	30.39	1.483	6.150	0.1503	0.387
"40"	38.5	0.541	1.223	31.59	30.25	7.541e	24.72	1.393	6.148	0.1506	0.388
"50"	47.8	0.541	1.519	31.79	30.18	7.466e	19.87	1.298	6.164	0.1506	0.388
Average									6.154		
10	38.6	0.541	1.23	28.53	27.16	7.47c	22.08	1.34	6.13	0.1478	0.385
11	39.1	0.541	1.24	29.42	27.98	7.46c	22.57	1.35	6.11	0.1436	0.379
14	41.5	0.541	1.32	31.35	29.94	7.51c	22.69	1.36	6.15	0.1497	0.387
15	36.3	0.541	1.15	28.24	27.00	7.52c	23.48	1.37	6.15	0.1423	0.377
Average									6.135		

about the right amount of Ca^{++} ion to satisfy both solubility products, there was but little precipitation of the calcium as carbonate. There was no difference in $pK'_{s.p.}CaCO_3$, but some in $pK'_{s.p.}Ca_3(PO_4)_2$, with difference in time of saturation from 1 day to 8 days.

Group II. Experiment 8.—The initial Ca^{++} ion concentration was quite high, $[HCO_3^-]$ and $[CO_3^{=}]$ normal, but no phosphate was initially in solution. At first the high $[Ca^{++}]$ caused precipitation of the bicarbonate as $CaCO_3$, and, as the Ca^{++} ion concentration decreased, the driving force of the reaction decreased. In the

TABLE VIII.

Solubility of $CaCO_3$ and $Ca_3(PO_4)_2$ in Salt Solutions Saturated at 38° with Both Solids.

Experiment No.	Saturation time.	Initial concentrations.			Final concentrations.							pK'$_{s.p.}$ $CaCO_3$	pK'$_{s.p.}$ $Ca_3(PO_4)_2$	$\sqrt{\mu}$	Reaction.	
		$[HCO_3^-]$ $\frac{M \times 10^3}{kg.\ H_2O}$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[Ca^{++}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	Total $[CO_2]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[HCO_3^-]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[CO_3^=]$ $\frac{M \times 10^3}{kg.\ H_2O}$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[PO_4^{\equiv}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[Ca^{++}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	pcH$^+$				
	hrs.															
9	20	30.27	None.	None.	31.04	29.43	0.129	0.275	1.05	0.31	7.43c	7.40	26.49	0.383	CO_3 ppt.	PO_4 diss.
14	8 days.	30.27	"	"	31.35	29.92	0.157	0.133	0.625	0.26	7.51c	7.39	27.17	0.387	"	"
8	20	30.27	None.	7.76	19.20	17.64	0.045	0.038	0.078	1.33	7.20c	7.23	26.86	0.378	CO_3 ppt.	PO_4 diss.
16	44	30.27	2.42	None.	33.62	32.08	0.165	1.06	4.86	0.25	7.50c	7.38	25.42	0.378	CO_3 diss.	PO_4 ppt.
24	7 days.	30.27	2.42	"	33.67	32.31	0.195	0.93	5.11	0.23	7.57c	7.35	25.50	0.380	"	"
5C	17	30.27	0.24	0.50	30.07	28.74	0.154	0.25	1.21	0.26	7.52c	7.40	26.61	0.398	CO_3 ppt.	PO_4 ppt.
B	18	30.27	1.21	2.52	27.53	26.53	0.188	0.332	2.18	0.228	7.64c	7.37	26.24	0.365	"	"
D	18	29.67	1.19	2.47	26.93	25.95	0.180	0.324	2.08	0.228	7.63c	7.39	26.28	0.365	"	"
7′	18	30.27	0.71	1.51	29.50	27.77	0.103	0.289	0.848	0.306	7.36g	7.49	26.69	0.379	"	"
7A′	18	32.80	0.71	1.51	32.21	30.40	0.118	0.302	0.960	0.273	7.38g	7.49	26.73	0.382	"	"
29	18	32.80	1.21	2.52	30.32	28.75	0.126	0.293	1.12	0.28	7.43c	7.45	26.55	0.393	"	"
5	20	35.32	1.21	4.65	29.24	27.87	0.136	0.325	1.41	0.25	7.48c	7.47	26.50	0.383	"	"
26	18	40.36	1.21	2.52	38.68	36.39	0.135	0.508	1.61	0.29	7.36c	7.41	26.20	0.401	"	"
20	68	40.36	1.21	2.52	38.06	36.60	0.242	0.397	2.42	0.17	7.61c	7.39	26.55	0.401	"	"
15	8 days.	40.36	1.21	7.76	28.24	27.00	0.145	0.063	0.303	0.43	7.52c	7.21*	27.15	0.377	"	"
25	18	40.36	1.21	2.52	38.07	35.73	0.127	0.677	2.04	0.19	7.34c	7.62*	26.54	0.401	CO_3 ppt.	PO_4 ppt.
21	68	40.36	1.21	2.52	41.52	38.03	0.093	0.444	0.85	0.42	7.18c	7.41	26.28	0.402	"	"
23	7 days.	30.27	2.42	None.	31.85	30.19	0.132	1.40	5.33	0.03	7.43c	8.40*	28.10*	0.379	CO_3 diss.	PO_4 ppt.
												7.43	26.53		Average of Groups IV and V.	

*Not figured in computing average.

meantime, $Ca_3(PO_4)_2$ dissolved in order to furnish $PO_4^{\equiv}$ ion to satisfy the $Ca_3(PO_4)_2$ stoichiometric solubility product. But in dissolving, it also furnished *more* Ca^{++} ions causing further precipitation of the carbonate and diminution of calcium. Bicarbonate was reduced, H_2CO_3 increased, and the solution became more acid. Apparently the reaction had not been completed in 20 hours, for a condition of slight supersaturation with respect to carbonate, and slight unsaturation with respect to phosphate, was obtained at the end of that period.

Group III. Experiments 16 and 24.—This group differs markedly from all of the others presented in this section in this respect: The solutions initially contained bicarbonate, carbonate, phosphate, but no calcium. In order to satisfy the equilibrium condition that the solution be saturated with respect to both solid phases, it was necessary that some Ca^{++} ion go into solution by dissolving $CaCO_3$ and $Ca_3(PO_4)_2$. The latter, furnishing $PO_4^{\equiv}$ ion to a solution already holding a large amount, dissolved but slightly, if at all. Hence $CaCO_3$ dissolved, furnishing HCO_3^-, and $CO_3^{=}$ ions, according to the reactions

$$(a)\quad CaCO_3 + H_2CO_3 \rightleftarrows Ca(HCO_3)_2 \rightleftarrows Ca^{++} + 2HCO_3^- \quad \Updownarrow \quad 2H^+ + 2CO_3^{=}$$

Also, $Ca_3(PO_4)_2$ may have reacted though slightly, as

$$(b)\quad Ca_3(PO_4)_2 + 2H_2CO_3 \rightleftarrows 2CaHPO_4 + Ca(HCO_3)_2$$
$$\Updownarrow \qquad\qquad \Updownarrow$$
$$2Ca^{++} + 2HPO_4^{=} \qquad Ca^{++} + 2HCO_3^-$$
$$\Updownarrow \qquad\qquad \Updownarrow$$
$$2H^+ + 2PO_4^{\equiv} \qquad 2H^+ + 2CO_3^{=}$$

Therefore reaction (*b*) was stopped with but little Ca^{++} in solution, and an increase in both $PO_4^{\equiv}$ and $CO_3^{=}$ ion, especially at slightly greater alkalinity ($p\alpha_{H^+}$ = 7.50 and 7.57). Since there was already sufficient $CO_3^{=}$ ion in solution to take care that $pK'_{s.p.}$ $CaCO_3$ was satisfied, the reaction (*a*) tended to the left. Since the solubility product of $Ca_3(PO_4)_2$ was greatly exceeded, reaction (*b*) also tended to run to the left, causing a decrease in $[Ca^{++}]$. However, such a decrease would have disturbed reaction (*a*) again, whereas the solubility product for $CaCO_3$ was almost at the equilibrium value. The rate of adjustment was apparently

too slow, the final result being a supersaturation of $Ca_3(PO_4)_2$. It may be noted that the final state of affairs for Experiment 24 after 7 days saturation was very closely approximated by the final result of No. 16, a similar experiment, which had been run only for a little less than 2 days.

Group IV.—All of the experiments in this group had initially in solution, calcium, phosphate, and bicarbonate. Hence all reactions were precipitation reactions and should have been fairly well complete in a short time, in the presence of the two solid phases. Results of this and of Group V show an average of $pK'_{s.p.}CaCO_3$ = 7.43 at an average ionic strength for which $\sqrt{\mu} = 0.387$. The experiment upon $CaCO_3$ and $Ca_3(PO_4)_2$ solubility with changing concentration indicated a value of 7.46 for $pK'_{s.p.}CaCO_3$ at this ionic strength. The corresponding average $pK'_{s.p.}Ca_3(PO_4)_2$ found was 26.53, the actual dilution curve at this ionic strength being at 26.67. Undoubtedly, the experiments in this group represent the best ones of simultaneous calcium carbonate and phosphate equilibria. Under these conditions, equilibrium is rapidly attained.

Examination of the data for all experiments of the five groups reveals no apparent correlation between the stoichiometric solubility products obtained, and variation in $p\alpha_{H^+}$, final composition, or time of saturation after 16 hours. Hence these results were considered as a correct basis for comparison with results to be given on work with biological fluids.

Table IX gives the corresponding results of Holt, La Mer, and Chown (p. 542) recalculated on the basis of the stoichiometric dissociation constants of phosphoric acid used in this work. By comparison with our own, we find the following for $pK'_{s.p.}$ $Ca_3(PO_4)_2$:

Author.	Serum salts.	NaCl curve.	$\sqrt{\mu}$
Holt, La Mer, and Chown	25.58	27.75	0.382
Sendroy and Hastings	26.53	26.67	0.387

As pointed out in a previous section, our results with artificial edema fluid check those with NaCl solutions quite well for $pK'_{s.p.}$ $Ca_3(PO_4)_2$ and $pK_1''H_2CO_3$. However, all of these experiments

were performed under very similar conditions. Holt, La Mer, and Chown obtained serum salt solubility products for $Ca_3(PO_4)_2$ at $p\alpha_{H^+}$ between 6.95 and 7.70. In this respect, their experiments are comparable with ours, and the discrepancy between them may be ascribed to the difference in saturation. One might expect the velocity of precipitation of $Ca_3(PO_4)_2$ in their experiments to be somewhat less than if the reaction had taken place in the presence of solid $Ca_3(PO_4)_2$. On the other hand, the discrepancy between their NaCl dilution curve and ours, and between

TABLE IX.

$pK'_{s.p.}$ $Ca_3(PO_4)_2$ at 38° in Serum Salt Solutions.

Recalculated from Holt, La Mer, and Chown (1925,*a*), Table IX, p. 542.

$Ca(OH)_2+H_3PO_4$, shaking 8 days.

Experiment No.	$p\alpha_{H^+}$	$[Ca^{++}]$ M × 10^3 kg. H_2O	Total $[PO_4]$ M × 10^3 kg. H_2O	$[H_2PO_4^-]$ M × 10^3 kg. H_2O	$[HPO_4^=]$ M × 10^3 kg. H_2O	$[PO_4^{\equiv}]$ M × 10^6 kg. H_2O	pCa^{++}	$pPO_4^{\equiv}$	$pK'_{s.p.}$ $Ca_3(PO_4)_2$	μ	$\sqrt{\mu}$
1	7.02	0.144	6.52	1.93	4.59	7.96	3.84	7.10	25.72	0.1487	0.386
2	6.95	0.126	6.36	2.15	4.21	6.22	3.90	7.21	26.12	0.1480	0.385
3	7.05	0.151	6.13	1.77	4.36	8.11	3.82	7.09	25.64	0.1493	0.386
4	7.05	0.124	5.91	1.71	4.20	7.82	3.91	7.11	25.95	0.1477	0.384
5	6.92	0.146	7.57	2.68	4.89	6.82	3.84	7.17	25.86	0.1462	0.382
6	7.22	0.154	4.55	0.98	3.57	9.82	3.81	7.01	25.45	0.1463	0.382
7	7.22	0.134	3.94	0.85	3.09	8.55	3.87	7.07	25.75	0.1451	0.381
8	7.29	0.096	3.23	0.61	2.62	8.50	4.02	7.07	26.20	0.1440	0.379
9	7.40	0.126	2.65	0.41	2.24	9.36	3.90	7.03	25.76	0.1431	0.378
10	7.10	0.154	4.45	1.18	3.27	6.83	3.81	7.17	25.77	0.1421	0.377
11	7.70	0.116	1.64	0.14	1.50	12.50	3.94	6.90	25.62	0.1410	0.376
Average									25.58		

it and their own serum salt experiments, is difficult to explain. It may be that our use of what may be uncertain values for the first or the third stoichiometric dissociation constant of phosphoric acid at such low $p\alpha_{H^+}$ results in the variation noted. The experiments on $Ca_3(PO_4)_2$ solubility alone in salt solutions, Paper II, all showed a higher figure for $pK'_{s.p.}Ca_3(PO_4)_2$. The reason for this is not clear. However, inasmuch as our interest is centered upon systems in equilibrium with both solids, it will be unnecessary to take into account the results of other systems at this time.

Solubility of Bone in Salt Solution.—In order to be able to draw conclusions as to the behavior of the bone salts from the foregoing experiments with solid $CaCO_3$ and $Ca_3(PO_4)_2$ *in vitro*, it was considered desirable to make certain that the carbonate and phosphate of bone behaved as did the isolated salts used in our experiments. Dried human femur bones were obtained and pulverized into a fine powder. The dried and decomposed organic matter, lipoids, etc., in the powder, was not separated in Experiments 25 and 21. In one experiment, No. 23, the material was thoroughly washed several times. Group V, Table VIII, gives the results obtained with artificial edema fluid when saturated with bone powder. Experiments 21 and 25 show little difference from the results obtained in Group IV. Experiment 23 seems to be very much like those of Table II, Paper II, where only calcium phosphate was used as the saturating body. It is not known whether the effect of thorough washing would cause the discrepancy noted, or not. One may conclude from these experiments, however, that the calcium salts of bone do not behave very differently from the inorganic salts $CaCO_3$ and $Ca_3(PO_4)_2$.

Solubility of Calcium Carbonate and Tertiary Calcium Phosphate in Serum.

In the previous section the conditions under which serum salt solutions could be brought into simultaneous equilibrium with $CaCO_3$ and $Ca_3(PO_4)_2$ have been described. Using the data so obtained, it was thought that we were in a better position to understand, if not to explain, the problem of serum calcium and its relation to the bone calcium.

By studying the reaction of serum when subjected to widely varying changes in its composition and condition, and by comparing its reaction to such changes with its normal behavior, an effort was made to arrive at a satisfactory explanation of the nature of the serum calcium and the manner in which it exercises its physiological function.

In such a study, we must realize that when one is dealing with serum it may or may not exhibit *in vitro* all or some of the properties that it possesses when in the body where it has the opportunity to react with other tissues. Nevertheless, realizing this limitation, on the basis of our experiments, we have been able to

arrive at some definite conclusions as to the nature of the calcium compounds of serum. The behavior of these compounds affords a reasonable, though only partial explanation of the physiological behavior of serum as a medium for transporting calcium.

Serum as drawn at normal $p\alpha_{H^+}$ had values of $p([Ca] \times [CO_3^{=}])$ or $pCaCO_3$ of 6.40, and of $p([Ca]^3 \times [PO_4^{\equiv}]^2)$ or $pCa_3(PO_4)_2$, of 22.50. After saturation with solid $CaCO_3$ at 38°, these values did not change for $p\alpha_{H^+} = 7.40$. In fact, there had been practically no change in total [Ca] or total [PO_4] over a wide $p\alpha_{H^+}$ range. After saturation with solid $Ca_3(PO_4)_2$, however, $pCaCO_3$ was 7.23 and $pCa_3(PO_4)_2$ was 24.77 at $p\alpha_{H^+}$ 7.37 (Table IV, Paper II). There had been a decided decrease in [Ca] from 3.19 to 0.62 mM per kilo of H_2O, while [PO_4] had remained almost constant. All of these values are lower than those for $pK'_{s.p.}CaCO_3$ (7.40) and $pK'_{s.p.}$(26.50) in serum salt solutions.

From thermodynamic considerations, it has been shown that, when a solution is in equilibrium with a solid salt such as $CaCO_3$, *it is a necessity* that the product of the activity of Ca^{++} ion times the activity of the $CO_3^{=}$ ion be a constant,—which is the activity solubility product. In terms of concentration, at equilibrium at any given ionic strength, $[Ca^{++}] \times [CO_3^{=}] = K'_{s.p.}CaCO_3$. Hence, if the $[CO_3^{=}]$ is fixed and known, and the stoichiometric solubility product at the given ionic strength is known, one may calculate for a system at equilibrium, the value for $[Ca^{++}]$.

It has been shown by Van Slyke and collaborators (1925) that the ionic strength of horse serum is about 0.167, when protein and other unknown anions are taken into consideration. For our purpose, it will be convenient here to assume $\mu = 0.160$ as a round figure, for *all* sera, the possible effect of protein being neglected for the time being. On comparing the values for salt solutions at this ionic strength, with those for serum at $p\alpha_{H^+} = 7.4$, we find in round numbers, at 38°,

(*a*) Salt solutions + $CaCO_3$, $pK'_{s.p.}$ $CaCO_3$ ..... = 7.4
(*b*) Serum as drawn, p $CaCO_3$ ..... = 6.4
(*c*) " + $CaCO_3$, p $CaCO_3$ ..... = 6.4
(*d*) Salt solutions + $Ca_3(PO_4)_2$ + $CaCO_3$ } $pK'_{s.p.}$ $Ca_3(PO_4)_2$ ..... = 26.5
(*e*) Serum as drawn, p $Ca_3(PO_4)_2$ ..... = 22.5
(*f*) " + $Ca_3(PO_4)_2$, p $Ca_3(PO_4)_2$ ..... = 24.7

Evidently, $pCaCO_3$ or $p([Ca] \times [CO_3^{=}])$ and $pCa_3(PO_4)_2$ or $p([Ca]^3 \times [PO_4^{\equiv}]^2)$ do not have the same signifiance in salt solutions as in serum, even when the latter is in equilibrium with the solid salt. This can only mean that at equilibrium of systems (*c*) and (*f*), all of the [Ca] is not in the form of *Ca^{++} ions*. Of course, for serum as drawn (*b*), the same conclusion would apply.

In arriving at this conclusion, several assumptions have been involved. The first is that the systems studied in the presence of the solid salts did come to equilibrium with the salt, regardless of what happened to the concentration of total calcium [Ca]. Even in the case of salt solutions containing phosphate which seemed to give high values for the solubility product of $CaCO_3$ in the presence of that salt, we are consistent with the above assumption, by assuming that the extra calcium was present, with phosphate in some form or other, *not* ionized. Thermodynamic considerations such as those on which this work is based make it extremely unlikely that the concentration of calcium ion in such a solution did not obey the rule expressed by equation (8) when the solid salt $CaCO_3$ was present. The criterion of equilibrium was the attainment of a constant value for $pK'_{s.p.}$. The minimum time for this under these conditions was not determined, but we are fairly certain that for most reactions our experiments were run long enough to rule this out as a factor.

Another assumption is that the activity coefficient of HCO_3^- and of $CO_3^{=}$ ion is the same in serum as in salt solutions. On the latter point, we have no data or direct evidence. However, as has been mentioned before, the activity coefficient of HCO_3^- ion in serum has already been found to be apparently almost identical with the same quantity in a salt solution of corresponding ionic strength. In the light of this, the assumption made with regard to $CO_3^{=}$ ion, does not seem an unreasonable one.

With these assumptions, one may proceed further. In such systems as may be considered in equilibrium with the solid, after having obtained the value for $[Ca^{++}]$, one may obtain [CaX] where, as has been mentioned before, [CaX] = total [Ca] − $[Ca^{++}]$. This does not, in any case, involve any explanation or assumption as to what CaX really is, other than that it is calcium apparently in solution not in the form of Ca^{++} ions.

Horse Serum Shaken with $CaCO_3$ and $Ca_3(PO_4)_2$ for Varying

TABLE X.

Effect of Shaking Horse Serum at 38° for Varying Lengths of Time, with Solid $CaCO_3$ and $Ca_3(PO_4)_2$.

Time.	$p\alpha_{H^+}$ (colorimetric).	Total $[CO_2]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[HCO_3^-]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[CO_3^=]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[Ca]$ $\frac{M \times 10^3}{kg.\ H_2O}$	pCa	$pCO_3^=$	$pCaCO_3$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[PO_4^{\equiv}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$pPO_4^{\equiv}$	$pCa_3(PO_4)_2$
0	7.46	29.68	28.21	0.132	3.19	2.50	3.88	6.38	0.79	3.25	7.49	22.48
5 min.	7.42	28.23	26.73	0.114	1.81	2.74	3.94	6.68	0.65	2.40	7.62	23.46
1 hr.	7.45	28.33	26.92	0.123	1.67	2.78	3.91	6.69	0.60	2.42	7.61	23.57
3 hrs.	7.45	27.95	26.56	0.121	1.67	2.78	3.92	6.70	0.59	2.37	7.63	23.59
22 "	7.23	22.03	20.37	0.056	1.49	2.83	4.25	7.08	0.35	0.77	8.11	24.72

Lengths of Time.—Although previous work showed that salt solutions could be placed in contact and brought into equilibrium with both solid $CaCO_3$ and $Ca_3(PO_4)_2$ by shaking or rotating for about 20 hours, it was thought desirable to attempt to verify this for serum. Holt, La Mer, and Chown, using sterile technique, shook serum with $Ca_3(PO_4)_2$ for from 8 to 10 days. Not only was this inconvenient, but such a procedure ran the risk of having some change, *e.g.* autolysis, occur in the serum thereby changing its character. If conditions could be adjusted so that the reaction was practically complete after 1 day, it would be a decided advantage. The results could then be compared with those of

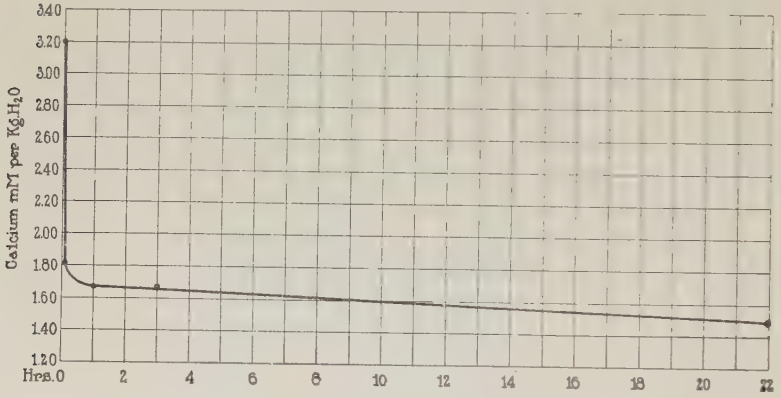


FIG. 4. The rate of precipitation of calcium from separated serum when it is saturated with solid $CaCO_3$ and $Ca_3(PO_4)_2$. Calcium in millimols per kilo of H_2O is plotted as ordinates and time in hours as abscissæ.

salt solution experiments performed under similar conditions. Furthermore, a study of the rate of change of calcium concentration would afford some indication of the mechanism of the reaction.

Accordingly, fresh horse serum from defibrinated blood was rotated at 38° with solid $CaCO_3$ and $Ca_3(PO_4)_2$; samples were removed at various intervals with the proper precautions and analyzed. Results are given in Table X and Fig. 4.

In contrast to the behavior of serum when saturated with only $CaCO_3$ as the solid phase, the total calcium decreased rapidly upon bringing the serum into contact with the solid phase. At first

sight, this would seem to be an argument for the theory of supersaturation with $Ca_3(PO_4)_2$. However, an examination of the change involved reveals that all of the calcium lost was precipitated not as phosphate, but mostly in the form of carbonate.

For example, in the first 3 hours of saturation there was a loss in bicarbonate and carbonate corresponding to the precipitation of 0.84 mM per kilo of H_2O of calcium as $CaCO_3$. The loss in total phosphate corresponded to the precipitation of 0.30 mM of calcium as $Ca_3(PO_4)_2$. By analysis, the actual loss in calcium was 1.52 mM, which is 0.38 mM more than can be accounted for from the decrease in bicarbonate, carbonate, and phosphate. From this experiment alone, one might infer that a condition of supersaturation with respect to both $CaCO_3$ and $Ca_3(PO_4)_2$ existed in the serum before equilibration with the solid.

This experiment is in agreement with the experimental results of Holt, La Mer, and Chown (see Table III, Paper II) on serum saturated with $Ca_3(PO_4)_2$, and with the results of Holt and his collaborators (1925), who saturated cerebrospinal fluid with $Ca_3(PO_4)_2$. These authors apparently overlooked the fact that the decrease in inorganic phosphate in their experiments was not sufficient to account for more than approximately 30 per cent of the decrease in calcium at $p\alpha_{H^+} = 7.40$. At greater alkalinity, more was lost as phosphate up to 52 per cent. It would seem, therefore, that a theory quantitatively accounting for the calcium in the blood must embrace other possibilities than only supersaturation with respect to $Ca_3(PO_4)_2$.

Does this experiment necessarily mean that serum is supersaturated with respect to $CaCO_3$? The experiments with only solid $CaCO_3$ would seem to be one argument against this possibility. It will be recalled that salt solutions containing phosphate after saturation with solid $CaCO_3$, still showed an apparent supersaturation with respect to $CaCO_3$. Upon adding $Ca_3(PO_4)_2$ also, a system was obtained, which soon attained equilibrium with both salts. The total calcium was decreased, but by theory, the *Ca^{++} ion* concentration in both systems before and after addition of $Ca_3(PO_4)_2$, must have been that demanded by equilibrium conditions.

Salt solutions containing sodium citrate showed what seemed to be a very high degree of supersaturation with respect to $CaCO_3$,

in the presence of the solid. Upon removing $CaCO_3$ and adding $Ca_3(PO_4)_2$ and saturating, some calcium was lost as the solution entered into equilibrium with the new phase. As a result, the system still showed a very high $pCaCO_3$ at a time when the solution was *unsaturated* with respect to $CaCO_3$.

Serum to which an excess of calcium had been added, retained this calcium and showed an apparent supersaturation of $CaCO_3$ even in the presence of the solid. Serum partially deprived of its calcium did not dissolve calcium on saturation with $CaCO_3$. On removal of phosphate, however, it was found possible to decrease or increase the total calcium even in the presence of solid $CaCO_3$.

In all of these conditions, the total [Ca] was always more than would be found in a simple salt solution of the same $[CO_3^{=}]$ after saturation with $CaCO_3$. Hence, it can be seen that in salt solutions it is possible under several different conditions for a system to be in equilibrium with solid $CaCO_3$, to show an apparent supersaturation with respect to $CaCO_3$, and to lose calcium upon being equilibrated with $Ca_3(PO_4)_2$. Hence, although $CaCO_3$ was precipitated from serum on adding solid $CaCO_3$ and solid $Ca_3(PO_4)_2$ in the light of its lack of response to $CaCO_3$ alone, we must conclude that its behavior in the case under consideration was due to the presence of the $Ca_3(PO_4)_2$ and its adjustment to the new solid phase, and not because it may have been supersaturated with respect to $CaCO_3$. It is highly improbable that any mechanical phenomena could have prevented the precipitation of $CaCO_3$ from a supersaturated solution in the presence of $CaCO_3$. Moreover, it is difficult to see how any such factor should have become inoperative on the addition of $Ca_3(PO_4)_2$.

Horse Blood Shaken with $CaCO_3$ and $Ca_3(PO_4)_2$ for Varying Lengths of Time.—In order to determine whether the presence of cells altered the reaction of serum toward solid $CaCO_3$ and $Ca_3(PO_4)_2$, freshly drawn defibrinated whole blood was brought into contact with both of these salts, samples were taken, and the serum separated and analyzed at various time intervals. It was anticipated that the presence of the cells might act to delay equilibrium, due to mechanical causes. When compared with the previous time experiment with separated serum, it is evident (Table XI, Fig. 5) that the cells exerted no appreciable influence

chemically or mechanically, on the final result with respect to $CaCO_3$. No analyses were made for phosphate. Inasmuch as the blood used here was not the same as that used for the serum experiment, a direct comparison cannot be made. The rate of precipitation of calcium, however, was apparently the same as for the previous experiment, most of the decrease taking place within the first 5 minutes. The zero point was assumed from other analyses of the same horse serum.

Effect of Shaking Untreated Horse Serum at 38° at Varying $p\alpha_{H^+}$.—In order to discover the effect on our systems, if any, of merely rotating serum at 38° for a period of about 16 hours, without any solid present, the experiment to be described was

TABLE XI.

Effect of Shaking Horse Blood at 38° for Varying Lengths of Time, with Solid $CaCO_3$ *and* $Ca_3(PO_4)_2$. *Serum Separated and Analyzed.*

Saturation time.	$p\alpha_{H^+}$ (colorimetric).	Total $[CO_2]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[HCO_3^-]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[CO_3^=]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[Ca]$ $\frac{M \times 10^3}{kg.\ H_2O}$	pCa	$pCO_3^=$	$pCaCO_3$
0	7.40*				3.30*	2.47	3.91*	6.38
5 min.	7.44	28.70	27.22	0.122	2.20	2.66	3.91	6.57
3 hrs.	7.38	26.59	25.09	0.098	1.49	2.83	4.01	6.84
20 "	7.32	22.50	21.09	0.071	1.31	2.88	4.15	7.03

* Assumed.

performed. Fresh horse serum was equilibrated at varying tensions of CO_2 to give the desired $p\alpha_{H^+}$ range. From the total CO_2 and the CO_2 tension $p\alpha_{H^+}$ was calculated from the curve for the ratio $\frac{pCO_2}{[CO_2]} = f_3$ at varying $p\alpha_{H^+}$, as described in the section on calculations. Results are recorded in Table XII.

The results indicate that shaking serum at 38°, in itself, has no effect on the amount of calcium held in solution, within a very wide range of $p\alpha_{H^+}$. Starting with a concentration of 3.40 mM Ca per kilo of H_2O, there was practically no change in this quantity from $p\alpha_{H^+}$ 6.27 to 7.95.

This experiment would suggest that whatever it is that keeps the calcium at a constant figure *in vivo* as far as change in $p\alpha_{H^+}$

is concerned, is still operative *in vitro*. Serum is apparently very well adapted to the biological function of keeping the calcium of the blood stream always at a constant level, within wide limits of variation of $[H^+]$, $[CO_3^{=}]$, and $[PO_4^{\equiv}]$.

Experiments given in Paper I in which $CaCO_3$ was present, under the same conditions, gave almost the identical curve for $pCaCO_3$ and $pCa_3(PO_4)_2$ with varying $p\alpha_{H+}$, as was given by serum rotated without solid salts. The immediate inference would be that serum in the body is in equilibrium with $CaCO_3$. However, it is known that under normal conditions in the body,

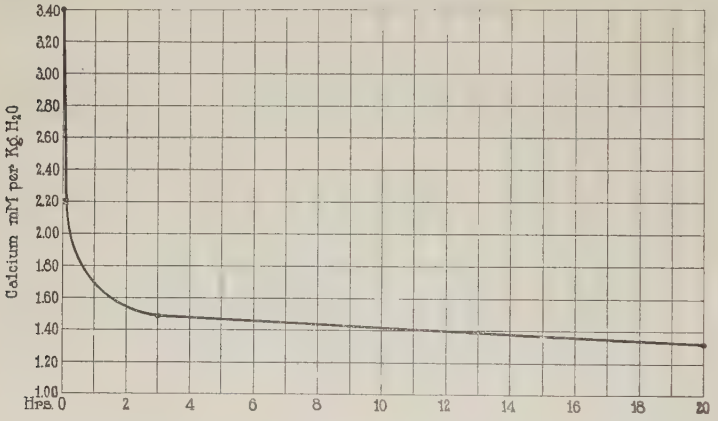


FIG. 5. The rate of precipitation of calcium from serum when whole blood is saturated with solid $CaCO_3$ and $Ca_3(PO_4)_2$. Calcium in millimols per kilo of H_2O is plotted as ordinates and time in hours as abscissæ.

the serum calcium is constant. Serum calcium *in vitro* has also been shown to be constant with change of $p\alpha_{H+}$, *when not in contact with* $CaCO_3$, and also to be unchanged when $CaCO_3$ was added. Hence, we cannot say that the serum was in equilibrium with $CaCO_3$ *in vivo* or *in vitro*.

Horse Serum Shaken at 38° with $CaCO_3$ and $Ca_3(PO_4)_2$ at Varying $p\alpha_{H+}$.—This experiment was performed in the same manner as the previous one without solids. The serum used had been standing in the ice box for a few hours before the experiment. The results obtained are given in Table XIII.

Here, the total calcium started with an initial concentration

TABLE XII.

Effect of Shaking Untreated Horse Serum at 38° for 16 Hours, at Varying $p\alpha_{H^+}$.

Experiment No.	$p\alpha_{H^+}$ (calculated).	Total $[CO_2]$ $\frac{M \times 10^3}{kg.\ H_2O}$	pCO_2 mm.	$[HCO_3^-]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[CO_3^=]$ $\frac{M \times 10^3}{kg.\ H_2O}$	[Ca] $\frac{M \times 10^3}{kg.\ H_2O}$	pCa	$pCO_3^=$	$pCaCO_3$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[PO_4^{\equiv}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$pPO_4^{\equiv}$	$pCa_3(PO_4)_2$
1	6.27	56.35	711.9	32.72	0.00997	3.35	2.48	5.00	7.48	0.692	0.0623	9.21	25.85
2	6.92	38.53	160.6	33.15	0.045	3.40	2.47	4.35	6.82	0.766	0.690	8.16	23.73
3	7.55	30.81	34.3	29.50	0.170	3.40	2.47	3.77	6.24	0.762	4.0	7.40	22.21
4	7.95	27.48	12.3	26.70	0.371	3.40	2.47	3.43	5.90	0.759	10.63	6.97	21.36
5	8.40	23.51	3.67	22.47	0.915	3.06	2.51	3.04	5.55	0.620	25.4	6.60	20.72
Original.						3.40							

TABLE XIII.

Effect of Shaking Horse Serum for 17 Hours at 38°, with Solid $CaCO_3$ and $Ca_3(PO_4)_2$.

Experiment No.	$p\alpha_{H^+}$ (calculated).	Total $[CO_2]$ $\frac{M \times 10^3}{kg.\ H_2O}$	pCO_2 mm.	$[HCO_3^-]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[CO_3^=]$ $\frac{M \times 10^3}{kg.\ H_2O}$	[Ca] $\frac{M \times 10^3}{kg.\ H_2O}$	pCa	$pCO_3^=$	$pCaCO_3$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[PO_4^{\equiv}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$pPO_4^{\equiv}$	$pCa_3(PO_4)_2$
1	6.25	52.45	682.6	29.77	0.00865	3.04	2.52	5.06	7.58	0.57	0.0472	9.33	26.21
2	6.85	31.28	150.4	26.66	0.030	1.32	2.88	4.52	7.40	0.544	0.381	8.42	25.48
3	7.21	26.49	62.1	24.34	0.064	0.98	3.01	4.19	7.20	0.62	1.30	7.87	24.80
4	7.80	21.78	13.52	21.12	0.215	1.18	2.93	3.67	6.60	0.876	8.58	7.07	22.92
5	8.17	18.84	5.14	18.23	0.439	1.19	2.92	3.36	6.28	0.887	21.3	6.67	22.10
Original as drawn.	7.35c	13.01	52.7 (Calculated.)	29.15	0.106	3.30	2.48	3.97	6.45	0.91	2.82	7.55	22.54
Original before saturation.....						3 03							

for [Ca] of 3.03 mM. This was maintained at $p\alpha_{H^+}$ 6.25, but *decreased* to 0.98, increasing again between $p\alpha_{H^+}$ = 7.21 and 7.80. This latter increase may be ascribed to the base-binding power of the proteins, which increases with $p\alpha_{H^+}$ causing a decrease in [HCO_3^-]. At low $p\alpha_{H^+}$, phosphate was lost even when calcium was not.

The important point is, that there was no evident precipitation of calcium as $Ca_3(PO_4)_2$. Some HCO_3^- was lost; some of it by precipitation, some due to the increase in base-binding power of the proteins. One may conclude from this experiment that supersaturation with respect to $Ca_3(PO_4)_2$ does not explain the decrease in [Ca] when $Ca_3(PO_4)_2$ is added to serum. Hence, supersaturation is not the hypothesis which explains or accounts for the existence of CaX. That portion of CaX which is left after serum has been rotated with $CaCO_3$ and $Ca_3(PO_4)_2$ increases with the $p\alpha_{H^+}$. This is in harmony with what one finds in a salt solution containing serum proteins, and is the result of the increased calcium power of the serum proteins with increasing $p\alpha_{H^+}$.

Horse Serum with Added $Ca(HCO_3)_2$ Rotated at 38° with $CaCO_3$ and $Ca_3(PO_4)_2$ at Varying $p\alpha_{H^+}$.—Previous experiments with $CaCO_3$ showed that serum could retain apparently in solution an excess of calcium added as $CaCl_2$, even in the presence of solid $CaCO_3$. This was also true when the added calcium was in the form of $Ca(HCO_3)_2$. To test this property in the presence of both $CaCO_3$ and $Ca_3(PO_4)_2$ as solid phases, calcium chloride was added to serum with an equivalent amount of $NaHCO_3$ to make the [Ca] concentration 7.41 mM per kilo of H_2O—more than double the original calcium content. The results are given in Table XIV.

Although phosphate was precipitated, as might be expected, in the presence of the solid phase and so much extra calcium, the total calcium also fell markedly; it did not reach the level of the previous experiment at any corresponding pH value; both [Ca] and [HCO_3^-] were high. This may be interpreted as an argument for the theory that $CaCO_3$ is in colloidal suspension or is attached to the surface of the proteins, or may mean the formation of a calcium-carbono-X compound, although there is nothing in the CO_2 figure to indicate this. It will be noticed that the $pCa_3(PO_4)_2$ value reached was near the value for the quantity in the

TABLE XIV.

Effect of Shaking Horse Serum at 38° for 17 Hours with $CaCO_3$ and $Ca_3(PO_4)_2$ after Addition of $Ca(HCO_3)_2$.

Experiment No.	$p\alpha_{H^+}$ (calculated).	Total $[CO_2]$ $M \times 10^3$ / kg. H_2O	pCO_2 mm.	$[HCO_3^-]$ $M \times 10^3$ / kg. H_2O	$[CO_3^=]$ $M \times 10^3$ / kg. H_2O	[Ca] $M \times 10^3$ / kg. H_2O	pCa	$pCO_3^=$	$pCaCO_3$	Total $[PO_4]$ $M \times 10^3$ / kg. H_2O	$[PO_4^{\equiv}]$ $M \times 10^3$ / kg. H_2O	$pPO_4^{\equiv}$	$pCa_3(PO_4)_2$
1	6.20	50.68	705.2	27.26	0.0071	5.40	2.27	5.15	7.42	0.362	0.0246	9.61	26.03
2	6.72	30.17	186.7	23.95	0.0204	4.91	2.31	4.69	7.00	0.164	0.0758	9.12	25.17
3	7.12	23.41	65.9	21.18	0.045	4.41	2.36	4.35	6.71	0.113	0.1831	8.74	24.55
4	7.52	21.37	25.4	20.42	0.110	3.01	2.52	3.96	6.48	0.154	0.739	8.13	23.82
Original.						3.30							
Reinforced.						7.41							

TABLE XV.

Effect of Shaking Horse Serum at 38° for 16 Hours with $CaCO_3$ and $Ca_3(PO_4)_2$ after Addition of Excess Phosphate.

Experiment No.	$p\alpha_{H^+}$ (calculated).	Total $[CO_2]$ $M \times 10^3$ / kg. H_2O	pCO_2 mm.	$[HCO_3^-]$ $M \times 10^3$ / kg. H_2O	$[CO_3^=]$ $M \times 10^3$ / kg. H_2O	[Ca] $M \times 10^3$ / kg. H_2O	pCa	$pCO_3^=$	$pCaCO_3$	Total $[PO_4]$ $M \times 10^3$ / kg. H_2O	$[PO_4^{\equiv}]$ $M \times 10^3$ / kg. H_2O	$pPO_4^{\equiv}$	$pCa_3(PO_4)_2$
1	6.18	50.38	718.7	26.51	0.00655	3.21	2.49	5.18	7.67	1.34	0.0844	9.07	25.62
2	6.75	29.68	174.5	23.86	0.022	1.52	2.82	4.66	7.48	1.31	0.655	8.18	24.83
3	7.19	28.44	68.4	26.11	0.065	1.02	2.99	4.19	7.18	1.34	2.68	7.57	24.11
4	7.77	22.48	15.2	21.77	0.207	0.81	3.09	3.68	6.77	1.26	11.41	6.94	23.16
5	8.15	19.74	56.1	19.12	0.434	0.73	3.14	3.36	6.50	1.25	28.23	6.55	22.52

previous experiment, whereas $pCaCO_3$ was lower than normal for these conditions.

Horse Serum Rotated at 38° with $CaCO_3$ and $Ca_3(PO_4)_2$ after the Addition of Excess Phosphate.—To normal horse serum was added a mixture of KH_2PO_4-Na_2HPO_4 of $p\alpha_{H+}$ = 7.40, sufficient to increase the phosphate content by 1.21 mM per kilo of H_2O. The normal horse serum value would be about 0.8 mM per kilo of H_2O. This serum was then rotated in contact with the two solid salts for 16 hours. The results are given in Table XV.

This experiment shows that calcium in serum is very easily precipitated in the presence of an excess of phosphate and the solid phase $Ca_3(PO_4)_2$. Moreover, examination of the phosphate figures reveals that although some phosphate had been precipitated, the amount in solution was almost constant at a high figure throughout a wide range of $p\alpha_{H+}$, whereas during this same period the calcium dropped steadily. Some of it was evidently precipitated as $CaCO_3$ since the loss in PO_4 cannot account at any $p\alpha_{H+}$ for the loss in calcium. In the presence of excess phosphate, the calcium precipitated at $p\alpha_{H+}$ 7.4 to a point corresponding to the amount accounted for by the protein and salt content of the serum. That is, at $p\alpha_{H+}$ 7.4, the [Ca] was the same as that of the experiment with two solids only.

By comparison with the previous experiment, it may be noted that the $pCaCO_3$ was lower in value throughout when excess $Ca(HCO_3)_2$ was present with the two solids, than when excess phosphate was added. The $pCa_3(PO_4)_2$ value for both conditions was very much the same. Hence some calcium phosphate must have been precipitated in the one case by excess calcium and in the other by excess phosphate. The carbonate concentration throughout being about the same for both experiments, the difference in calcium accounts for the difference in $pCaCO_3$ obtained.

This may possibly be regarded not as suggesting supersaturation with respect to $CaCO_3$ in the previous experiment, but as suggesting the presence of colloidal $CaCO_3$ in very fine particles in serum.

Horse Serum Rotated at 38° after Addition of Excess Phosphate.—This experiment, indicated in Table XVI, was similar to the one just described. PO_4 added was 2.25 mM per kilo of H_2O. However, no solid salts had been added. Under the circumstances,

TABLE XVI.

Effect of Shaking Horse Serum at 38° for 17 Hours after Addition of Excess Phosphate. No Solid Added.

Experiment No.	$p\alpha_{H^+}$ (calculated).	Total $[CO_2]$ $\frac{M \times 10^3}{kg.\ H_2O}$	pCO_2 mm.	$[HCO_3^-]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[CO_3^=]$ $\frac{M \times 10^3}{kg.\ H_2O}$	[Ca] $\frac{M \times 10^3}{kg.\ H_2O}$	pCa	$pCO_3^=$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[PO_4^{\equiv}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$pPO_4^{\equiv}$	$pCa_3(PO_4)_2$	$pCaCO_3$
1	6.17	48.20	696.4	25.08	0.00626	3.30	2.48	5.20	3.00	0.18	8.75	24.97	7.68
2	6.87	38.69	178.4	33.06	0.039	3.24	2.49	4.41	3.04	2.28	7.64	22.75	6.90
3	7.49	29.63	38.0	28.23	0.141	3.15	2.50	3.85	2.99	13.31	6.88	21.25	6.35
4	7.94	25.21	11.4	24.50	0.328	2.56	2.59	3.48	2.73	37.1	6.43	20.63	6.07
5	8.32	21.94	4.10	21.08	0.724	2.56	2.59	3.14	2.67	90.7	6.04	19.85	5.73

reasoning by analogy from the experiments done on salt solutions, one would expect the condition of supersaturation shown here. This experiment, although it shows that serum can be apparently supersaturated with respect to calcium phosphate, does not constitute an argument for the hypothesis that *normal* serum in the body is supersaturated with respect to $Ca_3(PO_4)_2$. The content of phosphate in normal horse serum is such that it is but little precipitated when $Ca_3(PO_4)_2$ is added. Hence the extra calcium must be due to something else.

Variation of Calcium Concentration with Dilution of Serum after Equilibrating with $CaCO_3$ and $Ca_3(PO_4)_2$.—In order to show that that portion of calcium which was left after precipitating calcium from serum, by shaking with $CaCO_3$ and $Ca_3(PO_4)_2$, was of a definite composition, the following experiment was performed. Five solutions were prepared in which the serum was diluted with a balanced salt solution, so that the concentration varied from that of 100 per cent serum to 100 per cent salt solution. This salt solution was of an ionic strength $\sqrt{\mu} = 0.398$ and contained $NaHCO_3$, NaCl, KCl, $CaCl_2$, $MgCl_2$, K_2SO_4, and Na_2HPO_4-KH_2PO_4 in the proportions generally found in serum. Hence it was the proteins and constituents of serum other than the salts, which were being diluted. Samples were saturated with the solid phases for 17 hours, then analyzed.

Due to the dilution of the serum proteins, $p\alpha_{H^+}$ was found more accurately for Experiments 3 and 4 by calculation on the basis of analyses of CO_2 content and CO_2 tension. By using the proper $\alpha^{\circ}_{CO_2}$ in accordance with the formula $\alpha^{\circ}_{CO_2} = 0.557 - 103\,\mu + 0.36\,P$, and assuming a protein content of 7 per cent in (100 per cent) serum, $[H_2CO_3]$ was calculated. From this value, and the total $[CO_2]$, the factor $f_2 = \frac{[H_2CO_3]}{[CO_2]}$ was obtained from which the $p\alpha_{H^+}$ was calculated.

Values of 6.13 and 9.79 for pK_1'' and pK_2' respectively, were used throughout.

By taking into account the water content of the solutions, the protein percentage per kilo of H_2O was calculated. Table XVII gives the results of this experiment, and indicates that the total calcium is proportional to the amount of serum. The results are not strictly comparable due to differences in $p\alpha_{H^+}$.

This seems to prove that that portion of calcium which is still left, at least, has a definite composition. As has been pointed out, this is probably a calcium-protein compound.

These results agree with previous experiments in which serum was so diluted and saturated with $CaCO_3$. Other experiments in which the amount of dialyzed serum protein was varied, also showed a proportionality of serum protein to calcium.

Variation in Vitro of $pCaCO_3$ and $pCa_3(PO_4)_2$ with $p\alpha_{H^+}$ in Serum of Normal and Pathological Humans.—All of our *in vitro* experiments having been performed on horse serum, it was thought interesting to compare some results with the serum of normal and pathological human individuals. Table XVIII and Figs. 6 and 7 indicate the results obtained by varying the $p\alpha_{H^+}$

TABLE XVII.

Variation of Calcium with Varying Serum Concentration, after Shaking 17 Hours at 38°, with $CaCO_3$ and $Ca_3(PO_4)_2$.

Experiment No.	$p\alpha_{H^+}$	Total $[CO_2]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[CO_3^=]$ $\frac{M \times 10^3}{kg.\ H_2O}$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[PO_4^{\equiv}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$\frac{[Ca]\ M \times 10^3}{kg.\ H_2O}$ (observed.)	Per cent serum per kg. H_2O.
1	7.27c	28.61	0.080	0.394	0.98	1.30	100.0
2	7.38c	28.58	0.105	0.358	1.20	0.98	74.1
3	7.43g	28.70	0.119	0.384	1.46	0.63	48.7
4	7.44g	29.82	0.126	0.326	1.27	0.49	24.0
5	7.57c	30.07	0.154	0.250	1.21	0.26	0.0

of serum within physiological limits. No change, or very little, took place in either total phosphate or calcium, but bicarbonate as usual decreased with increase in $p\alpha_{H^+}$, due to the increase in base-binding power of the proteins.

The average value for $pCaCO_3$ at $p\alpha_{H^+}$ 7.40 was found to be 6.49, and for $pCa_3(PO_4)_2$, 22.42. The latter values are in agreement with those obtained on normal untreated horse serum at this $p\alpha_{H^+}$, and with Holt, La Mer, and Chown's values given in Table III, Paper II, before shaking with $Ca_3(PO_4)_2$.

The average decrease in $pCaCO_3$ with $p\alpha_{H^+}$ was found to be 0.90 between $p\alpha_{H^+}$ 7.2 and 7.5. This is really $\Delta pCO_3^=$ variation with $p\alpha_{H^+}$ and hence can be used to calculate the value of $pCaCO_3$ for untreated serum, from any determined $p\alpha_{H^+}$ to some set

TABLE XVIII.

Variation in $pCaCO_3$ and $pCa_3(PO_4)_2$ of Serum in Vitro, with $p\alpha_{H^+}$. Human Serum of Normals and Pathological Cases.

Experiment No.	Condition.	$p\alpha_{H^+}$ (electrometric).	Total $[CO_2]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[HCO_3^-]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[CO_3^=]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[Ca]$ $\frac{M \times 10^3}{kg.\ H_2O}$	Total $[PO_4]$ $\frac{M \times 10^3}{kg.\ H_2O}$	$[PO_4^{\equiv}]$ $\frac{M \times 10^3}{kg.\ H_2O}$	pCa	$pCO_3^=$	$pCaCO_3$	$pPO_4^{\equiv}$	$pCa_3(PO_4)_2$	At $p\alpha_{H^+} = 7.40$ (calculated). $pCaCO_3$	At $p\alpha_{H^+} = 7.40$ (calculated). $pCa_3(PO_4)_2$
H1	Normal.	7.51	29.77	28.42	0.149	2.97			2.53	3.83	6.36				
H2		7.31	32.77	30.67	0.101	2.97			2.53	4.00	6.33			6.45	
O1	"	7.44	25.59	24.29	0.109	2.90	1.24	4.85	2.54	3.96	6.50	7.31	22.24		
O2		7.23	28.25	26.11	0.072	2.85	1.24	2.77	2.54	4.14	6.69	7.56	22.77	6.54	22.34
W1	"	7.50	30.83	29.42	0.151	2.63	1.29	5.90	2.58	3.82	6.40	7.23	22.20		
W2		7.31	33.42	31.29	0.103	2.66	1.29	3.57	2.58	3.99	6.57	7.45	22.64	6.49	22.43
H1	Pneumonia.	7.52	31.98	30.58	0.164	3.08	0.96	4.63	2.51	3.79	6.30	7.33	22.19		
H2		7.32	34.63	32.46	0.110	3.08	0.96	2.74	2.51	3.96	6.47	7.56	22.65	6.40	22.47
M1	"	7.49	29.23	27.90	0.140	2.98	1.29	5.77	2.53	3.85	6.38	7.24	22.07		
M2		7.29	31.75	29.62	0.093	3.00	1.33	3.50	2.52	4.03	6.55	7.46	22.48	6.46	22.26
G1	Cardiac.	7.55	33.22	31.83	0.183	2.72	1.31	6.83	2.57	3.74	6.31	7.17	22.05		
G2	decompensation.	7.37	36.21	34.11	0.130	2.66	1.31	4.27	2.58	3.89	6.47	7.37	22.48	6.45	22.41
V1	Chronic glomerular	7.51	29.53	28.20	0.148	2.44	1.30	6.11	2.61	3.83	6.44	7.21	22.25		
V2	nephritis.	7.30	31.37	29.30	0.094	2.40	1.28	3.45	2.62	4.03	6.65	7.46	22.78	6.55	22.52
C1	" "	7.55	30.33	29.07	0.167	2.35	1.34	6.98	2.63	3.78	6.41	7.16	22.21		
C2		7.35	32.33	30.39	0.110	2.28	1.37	4.23	2.64	3.96	6.60	7.37	22.66	6.55	22.55
Average														6.49	22.42
Range														6.40–6.55	22.26–22.55

reference $p\alpha_{H^+}$, as 7.40. Correspondingly, $\frac{\Delta pCa_3(PO_4)_2}{\Delta p\alpha_{H^+}}$ was found to be on the average – 2.35.

Since there was no solid phase present, we cannot calculate [Ca^{++}] with any degree of certainty. However, if we *assume*

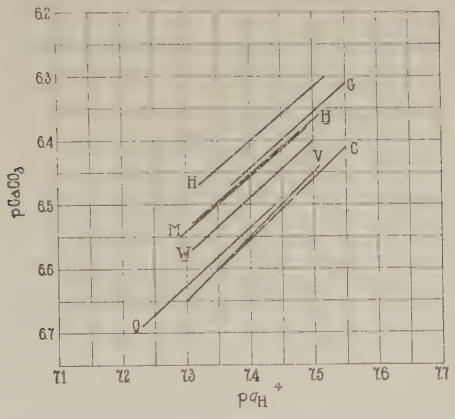


FIG. 6. The variation of $pCaCO_3$ with $p\alpha_{H^+}$ in normal and pathological humans. The letters designate the subjects. See Table XVIII.

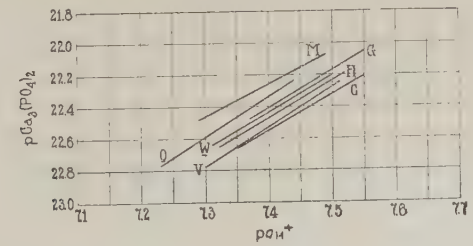


FIG. 7. The variation of $pCa_3(PO_4)_2$ with $p\alpha_{H^+}$ in normal and pathological humans. The letters designate the subjects. See Table XVIII.

equilibrium with $CaCO_3$, we find that at $p\alpha_{H^+}$ = 7.40 the amount of calcium over and above the amount in solution in a salt solution of the same ionic strength and same $CO_3^=$ ion concentration would constitute about 85 per cent of the total calcium in these sera. Regardless of what this may be due to, it is an

index of the extent to which serum differs from a salt solution in its ability to transport the element calcium. The different levels of total calcium at the same $p\alpha_{H^+}$ may be ascribed to variation in any of the calcium-binding constituents of serum.

Solubility of Calcium Salts in Vivo in Serum of Pathological Humans.—It was thought interesting to determine whether one could form any conclusions as to the variation in calcium of the serum in various diseases. A few cases were studied. Blood was drawn under oil with proper precautions to prevent loss of CO_2, allowed to clot, and centrifuged within $\frac{1}{2}$ hour, then transferred to

TABLE XIX.

$pCaCO_3$ and $pCa_3(PO_4)_2$ in Pathological Serum as Drawn.

Subject.	Date.	$p\alpha_{H^+}$	Total $[CO_2]$ m × 10^3 kg. H_2O	$[HCO_3^-]$ m × 10^3 kg. H_2O	$[CO_3^=]$ m × 10^3 kg. H_2O	Total $[PO_4]$ m × 10^3 kg. H_2O	$[PO_4^{\equiv}]$ m × 10^3 kg. H_2O	[Ca] m × 10^3 kg. H_2O	$pCaCO_3$	$pCa_3(PO_4)_2$
C (Control.)	Jan. 11	7.34c	27.88	26.19	0.093	1.73	5.20	2.12	6.70	22.58
	" 12					1.71		2.38		
	" 13	7.23c	27.99	25.88	0.071	1.39	3.10	2.28	6.79	22.94
	" 14	7.23c				1.49	3.32	2.43		22.79
	" 16	7.35e	27.50	25.86	0.094	1.55	4.78	2.61	6.61	22.38
	" 19	7.34e	30.98	29.10	0.103	1.92	5.78	3.35	6.46	21.92
J		7.11	9.59	8.67	0.018	7.92	12.51	1.94	7.46	21.94
O		7.42	25.80	24.43	0.104	2.34	8.68	2.23	6.63	22.07
F		7.33	23.49	22.03	0.076	1.93	5.65	2.20	6.78	22.48
M		7.22e	15.14	13.97	0.038	3.50	7.59	1.45	7.26	22.76

containers over mercury. Analyses were made for $p\alpha_{H^+}$, CO_2, Ca, and total PO_4. The results are given in Table XIX.

A most interesting case is that of Subject J (chronic glomerular nephritis) who showed a very low bicarbonate, very high phosphate, and slightly low calcium in serum. Analysis would indicate that the calcium was on the salt level ($pCaCO_3 = 7.46$) none being bound by protein or the hypothetical X. However, a calculation of $pCa_3(PO_4)_2$ on the basis of the phosphate content would indicate a very high value for CaX.

If CaX were due to supersaturation of $Ca_3(PO_4)_2$ or $CaHPO_4$ or some form of calcium combined with phosphate, the real value of $p([Ca] \times [CO_3^=])$ would be even greater, indicating a high degree

of unsaturation for $CaCO_3$. However, not knowing the Ca^{++} ion concentration, we have but little indication of what has taken place in a case like this.

The serum *in vivo* was unable to make any quick response to the disturbed $CaCO_3$ equilibrium. In this case, not only was there a diminution in calcium proteinate, and perhaps CaX, but the symptoms of convulsions, indicating a disturbed condition of nerve and muscle irritability, suggested that the Ca^{++} ion concentration had decreased, the mechanism whereby the calcium is kept constant having failed to make the proper adjustments.

Table XIX also contains the results of the study of a case of glomerular nephritis (Subject C) made at this hospital in collaboration with Dr. J. F. McIntosh. The patient was treated daily for 1 week with parathyroid hormone injections, in an effort to relieve a persistent edema. No relief was obtained, but the results proved to be interesting from the standpoint of the therapy involved. During the injections there was a fall in $p\alpha_{H^+}$, a progressive increase in calcium, while phosphate remained persistently high. It is evident that the hormone caused an increase in total calcium. A change occurred in blood, whereby the factor which we call CaX was probably increased.

DISCUSSION.

In presenting the evidence, we have shown that the theory of complete dissociation of electrolytes, on the basis of the Debye-Hückel theory, seems to fail as an adequate explanation for the solubility of calcium in serum. If this theory were to serve, it would be necessary to show that serum was supersaturated with calcium carbonate, or calcium phosphate, or both; or that the activity coefficients of calcium carbonate and of calcium phosphate are lower in serum than those found to obtain in serum salt solutions.

In our calculations, we have assumed that the activity coefficient of $CO_3^{=}$ ion is the same in serum as in salt solutions. It has been pointed out that there is no direct evidence for this. In the same way, the activity coefficient of Ca^{++} ion may be different in serum from what it is in salt solutions. However, even if we were to assume an effect of serum on the activity coefficients of Ca^{++}, $CO_3^{=}$, and $PO_4^{\equiv}$ ions to be identical, we would still not obtain values

for $\gamma_{CO_3^=}$, $\gamma_{PO_4^{\equiv}}$ and $\gamma_{Ca^{++}}$ which would explain the facts. Apparently, γ_{CaCO_3} would be 0.08 in serum instead of 0.26. We have no evidence at the present time, although such evidence may be produced in the future, that such specific ion effects may be attributed to the serum proteins.

Evidence has been produced which suggests that some portion of the serum calcium is bound by the proteins. However, in view of our lack of knowledge of the possible effect of the ionic strength of proteins in serum on the activity coefficients of the ions of difficultly soluble salts, we may only suggest what seems to be the probable magnitude of the calcium existing in the form of a protein compound. Apparently, this quantity is the amount that is left after saturating normal serum with solid calcium carbonate and tertiary calcium phosphate. It corresponds to a certain portion of what we call calcium unknown (CaX) in serum as drawn. Concerning the hypothesis of supersaturation, it may be stated that although such a condition may artificially be produced in salt solutions and in serum, the evidence in every direction seems to be strongly opposed to this point of view; at least, as the sole explanation for the apparently abnormal amount of calcium in serum.

Certainly, the experiments with $CaCO_3$ show no evidence of supersaturation. It has been shown that what apparent supersaturation of $Ca_3(PO_4)_2$ does sometimes occur, falls far short of accounting for the amount of extra calcium that exists apparently in solution in serum. In view of the experiments of Holt on cerebrospinal fluid saturated with $Ca_3(PO_4)_2$ and of others, the evidence seems strongly in favor of a diffusible, slightly ionized calcium compound. Our experiments with citrate certainly indicate that such a substance could behave the way serum calcium does. The change of $pCaCO_3$ and the change of $pCa_3(PO_4)_2$ with $p\alpha_{H^+}$ and with serum concentration, are points in favor of this hypothesis.

Although the evidence of experiments with $Ca_3(PO_4)_2$ and with $CaCO_3$ and $Ca_3(PO_4)_2$ apparently shows that the fall in calcium is due to the presence of $Ca_3(PO_4)_2$, we can only conclude, from the data, that the sole explanation is not supersaturation. As to exactly what happens when such large amounts of calcium are precipitated, we have no idea. The nature of the substance that

releases calcium as readily as our time experiments indicate, is unknown, and may only be the subject of speculation. Perhaps it is some slightly dissociable phosphate compound which holds calcium in unionized form.

It has been interesting to note, in this connection, that we also possess, in the active principle of the parathyroid gland, a sub-

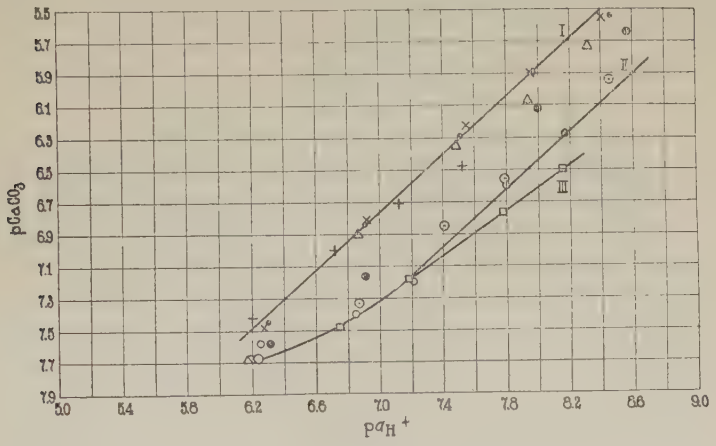


FIG. 8. The variation of $pCaCO_3$ with $p\alpha_{H^+}$ in horse serum under different experimental conditions. The points corresponding to the different experiments are designated as follows:

Symbol.	Experimental condition.	
•	With solid $CaCO_3$.	Curve I.
×	Without any solid.	Curve I.
○	With solid $CaCO_3$ and $Ca_3(PO_4)_2$.	Curve II.
+	" excess Ca and HCO_3, and with solid $CaCO_3$ and $Ca_3(PO_4)_2$.	
△	" " PO_4 " no solid.	
□	" " " " with solid $CaCO_3$ and $Ca_3(PO_4)_2$.	Curve III.
⊙	" solid $CaCO_3$ after depletion of calcium in solution.	
●	" " " and parathormone after depletion of calcium in solution.	

stance of biological origin, which can apparently hold calcium in solution, in much the same manner as do citrate solutions. It is highly improbable, however, that it can accomplish this alone and unassisted, in the body.

Concerning the question of equilibrium, it may be stated that the conditions for it have been obtained in our salt solution experi-

ments, and verified in those with serum at least with respect to $Ca_3(PO_4)_2$. Taking into consideration, the work of Holt, La Mer, and Chown on this question, together with our own evidence, it seems very improbable that any possible error in attainment of equilibrium could be of magnitude sufficiently great to affect any of the conclusions we have drawn.

The experiments presented, have, in large measure, been repeated often. Although they do not offer a complete solution to

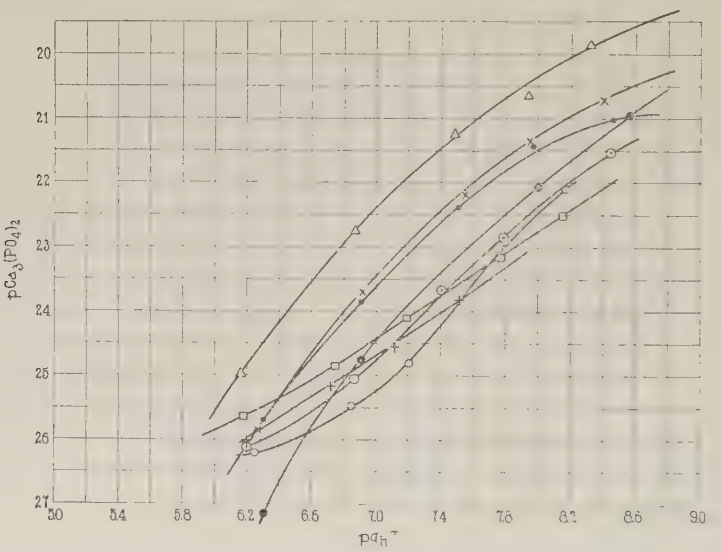


FIG. 9. The variation of $pCa_3(PO_4)_2$ with $p\alpha_{H^+}$ in horse serum under different experimental conditions. For explanation of symbols see legend to Fig. 8.

the problem of the nature of the calcium compounds of blood, the study of the solubility of the difficultly soluble salts has helped to increase our knowledge of the fitness of the mechanism which seems to be involved.

This work, together with what other evidence is available at the present time, indicates that we are dealing with a complex system adjusted to function, under normal conditions, in such a manner as to furnish the organism with the optimum quantity of calcium in ionized and unionized form, wherever and whenever it is needed.

The properties of serum, in this connection, seem to conform to the demands of such a system. While it is realized that we have but a small part of the physiological picture, inasmuch as most of our work has been done *in vitro*, our experiments are sufficiently clear in this respect; namely, that they indicate how calcium may be transported from one part of the body to the other.

For convenience, we have plotted in Figs. 8 and 9 the variation of $pCaCO_3$ and $pCa_3(PO_4)_2$ with $p\alpha_{H^+}$ under the various conditions for which they have been determined.

SUMMARY.

1. The solubility of $CaCO_3$ and $Ca_3(PO_4)_2$ in systems in equilibrium with both salts has been studied, and the conditions for equilibrium determined.

2. The stoichiometric solubility product of calcium carbonate has been found to vary with the ionic strength according to the equation $pK'_{s.p.}CaCO_3 = 8.58 - \frac{4.94\sqrt{\mu}}{1+1.85\sqrt{\mu}}$ at 38°.

3. The stoichiometric solubility product of tertiary calcium phosphate has been found to vary with the ionic strength according to the equation $pK'_{s.p.}Ca_3(PO_4)_2 = 30.95 - \frac{17.40\sqrt{\mu}}{1+1.48\sqrt{\mu}}$ at 38°.

4. The activity coefficient of Ca^{++} ion has been calculated from the solubility product data and the above equations.

5. The results indicate that these systems behave in conformity with the Debye-Hückel theory of strong electrolytes.

6. The solubility of calcium carbonate and tertiary calcium phosphate in balanced salt solutions under various conditions, has been determined.

7. The solubility of bone in salt solutions has been determined and found to be the same as that of its calcium salts.

8. The apparent solubility of calcium carbonate and tertiary calcium phosphate in serum has been studied under varying $p\alpha_{H^+}$ and time of saturation. Similar experiments without solids have been performed.

9. The solubility of these salts has been found to be unaffected *in vitro* by the presence of red blood cells.

10. The solubility of the salts has been found, after shaking,

with both salts, to be directly proportional to the serum concentration.

11. The variation *in vitro* of $pCaCO_3$ and $pCa_3(PO_4)_2$ with $p\alpha_{H^+}$ in serum of normal and pathological humans has been studied.

12. The $pCaCO_3$ and $pCa_3(PO_4)_2$ of serum as drawn was determined in certain pathological cases.

13. The biological significance of these results has been discussed. The data indicate that the theory of supersaturation is inadequate; that calcium exists in serum in abnormal amounts bound to some substance or substances which hold it in solution in unionized form.

Austin, J. H., Cullen, G. E., Hastings, A. B., McLean, F. C., Peters, J. P., and Van Slyke, D. D., *J. Biol. Chem.*, 1922, liv, 121.
Abbott, G. A., and Bray, W. C., *J. Am. Chem. Soc.*, 1909, xxxi, 729.
Barillé, A., *J. pharm. et chim.*, 1910, ii, 342.
Bell, R. D., and Doisy, E. A., *J. Biol. Chem.*, 1920, xliv, 55.
Benedict, S. R., and Theis, R. C., *J. Biol. Chem.*, 1924, lxi, 63.
Blanc, E., *J. chim. phys.*, 1920, xviii, 28.
Bosányi, A., *Am. J. Dis. Child.*, 1925, xxx, 780.
Briggs, A. P., *J. Biol. Chem.*, 1922, liii, 13.
Brönsted, J. W., and La Mer, V. K., *J. Am. Chem. Soc.*, 1924, xlvi, 555.
Brydges, R., *J. Chem. Soc.*, 1911, xcix, 125.
Buell, M. V., *J. Biol. Chem.*, 1923, lvi, 97.
Cameron, A. T., and Moorehouse, V. H. K., *J. Biol. Chem.*, 1925, lxiii, 687.
Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, lxiii, 461.
Clark, W. M., The determination of hydrogen ions, Baltimore, 2nd edition, 1922.
Collip, J. B., *J. Biol. Chem.*, 1925, lxiii, 395.
Collip, J. B., and Clark, E. P., *J. Biol. Chem.*, 1925, lxiv, 485; 1925, lxvi, 133.
Collip, J. B., Clark, E. P., and Scott, J. W., *J. Biol. Chem.*, 1926, lxiii, 439.
Debye, P., and Hückel, E., *Physik. Z.*, 1923, xxiv, 185.
Goodwin, H. W., and Robison, R., *Biochem. J.*, 1924, xviii, 1161.
Goto, K., *J. Biol. Chem.*, 1918, xxxvi, 355.
Greenberg, D. M., and Schmidt, C. L. A., *J. Gen. Physiol.*, 1926, viii, 271.
Greenwald, I., and Gross, J., *J. Biol. Chem.*, 1925, lxvi, 185, 217.
Harned, H. S., Treatise on physical chemistry, New York, 1924, ii, 701.
Hastings, A. B., and Sendroy, J., Jr., *J. Biol. Chem.*, 1924, lxi, 695; 1925, lxv, 445.
Holt, L. E., Jr., *J. Biol. Chem.*, 1925, lxiv, 579; lxvi, 23.
Holt, L. E., Jr., La Mer, V. K., and Chown, H. B., *J. Biol. Chem.*, 1925, lxiv, 509, 567.
Howland, J., and Kramer, B., *Tr. Am. Pediat. Soc.*, 1922, xxxiv, 204; *Monatschr. Kinderheilk.*, 1923, xxv, 279.
Hückel, E., *Physik. Z.*, 1925, xxvi, 93.
Hulett, G. A., and Bonner, W. D., *J. Am. Chem. Soc.*, 1909, xxxi, 390.
Hulett, G. A., and Menchin, H. D., *Physic. Rev.*, 1905, xxi, 388.
Kay, H. D., and Robison, R., *Biochem. J.*, 1924, xviii, 755.
Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, xlvii, 475.
Kugelmass, I. N., and Shohl, A. T., *J. Biol. Chem.*, 1923–24, lviii, 649.
La Mer, V. K., in Eucken, A., translated and adapted by Jette, E. R., and La Mer, V. K., Fundamentals of physical chemistry, New York, 1925.
Leather, J. W., and Sen, J. N., *Mem. Dept. Agric. India, Chem. Series*, 1909, i, 137; 1914, iii, 205.
Lewis, G. N., and Randall, M., *J. Am. Chem. Soc.*, 1921, xliii, 1112; Thermodynamics and the free energy of chemical substances, New York and London, 1923.

Martland, M., Hausman, F. S., and Robison, R., *Biochem. J.*, 1924, xviii, 1152.
Mathews, A. P., Physiological chemistry, New York, 3rd edition, 1921.
Michaelis, L., and Garmendia, P., *Biochem. Z.*, 1914, lxvii, 431.
Michaelis, L., and Kruger, R., *Biochem. Z.*, 1921, cxix, 307.
Neuhausen, B. S., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1922, liii, 365.
Noyes, A. A., and Eastman, G. W., *Carnegie Institution of Washington, Pub. No. 63*, 1907, 262.
Prideaux, E. B. R., and Ward, A. T., *J. Chem. Soc.*, 1924, cxxv, 423.
Robison, R., *Biochem. J.*, 1923, xvii, 286.
Robison, R., and Soames, K. M., *Biochem. J.*, 1924, xviii, 740.
Rona, P., and Takahashi, D., *Biochem. Z.*, 1913, xlix, 370.
Salvesen, H. A., and Linder, G. C., *J. Biol. Chem.*, 1923–24, lviii, 617, 635.
Sherman, H. C., The chemistry of food and nutrition, New York, 2nd edition, 1921.
Shipley, P. G., Kramer, B., and Howland, J., *Am. J. Dis. Child.*, 1925, xxx, 37.
Sörensen, S. P. L., and Linderström-Lang, K., *Compt. rend. trav. Lab. Carlsberg*, 1924, xv, No. 6.
Stanford, R. V., and Wheatley, A. H. M., *Biochem. J.*, 1925, xix, 697.
Tisdall, F. F., *J. Biol. Chem.*, 1923, lvi, 439.
Van Slyke, D. D., *J. Biol. Chem.*, 1918, xxxvi, 351.
Van Slyke, D. D., Hastings, A. B., Murray, C. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1925, lxv, 701.
Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, lxi, 523.
Watt, J. C., *Biol. Bull.*, 1923, xliv, 280.
Wells, H. G., *J. Med. Research*, 1906, xiv, 491.

Vita. Julius Sendroy, Jr., was born September 26, 1900, in Zombor, Hungary, and entered the United States in the following year. His grammar school education was obtained in the public schools of New York City, his secondary school work being done at Townsend Harris Hall, from which institution he was graduated in 1917. He attended the Evening Session of the College of the City of New York, from 1917 to 1923, receiving the degree of B.S. During the summer of 1921 and since 1923, he has studied at Columbia University. In 1925, he received the degree of M.A. Since 1920 he has been a technician in Chemistry with Dr. Donald D. Van Slyke at the Hospital of The Rockefeller Institute.

PUBLICATIONS.

Studies of gas and electrolyte equilibria in blood. VII. The effect of carbon monoxide on the acidity of hemoglobin. (With Hastings, A. B., Murray, C. D., and Heidelberger, M.) *J. Biol. Chem.*, 1924, lxi, 317.

Studies of acidosis. XX. The colorimetric determination of blood pH at body temperature without buffer standards. (With Hastings, A.B.) *J. Biol. Chem.*, 1924, lxi, 695.

Studies of acidosis. XXI. The colorimetric determination of the pH of urine. (With Hastings, A. B., and Robson, W.) *J. Biol. Chem.*, 1925, lxv, 381.

The effect of variation in ionic strength on the apparent first and second dissociation constants of carbonic acid. (With Hastings, A. B.) *J. Biol. Chem.*, 1925, lxv, 445.

Studies of gas and electrolyte equilibria in blood. VIII. The distribution of hydrogen, chloride, and bicarbonate ions in oxygenated and reduced blood. (With Van Slyke, D. D., Hastings, A. B., and Murray, C. D.) *J. Biol. Chem.*, 1925, lxv, 701.

THE WAVERLY PRESS, INC.
BALTIMORE, U.S.A.